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Frontiers in Life Science

Volume IV

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PREFACE

Life Sciences have always been a fundamental area of science. The exponential increase in the quantity of scientific information and the rate, at which new discoveries are made, require very elaborate, interdisciplinary and up-to-date information and their understanding. Enhanced understanding of biological phenomenon incorporated with interdisciplinary approaches has resulted in major breakthrough products for betterment of society. To keep the view in mind we are delighted to publish our book entitled ***"Frontiers in Life Science Volume IV"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of basic and applied life science.*

This book is published in the hopes of sharing the new research and findings in the field of life science subjects. Life science can help us unlock the mysteries of our universe, but beyond that, conquering it can be personally satisfying. We developed this digital book with the goal of helping people achieve that feeling of accomplishment.

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, India for taking pains in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editorial Team

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INCORPORATION OF RIPE BAEL FRUIT INTO CHOCOLATE AND ANALYSIS OF ITS TEXTURAL, SENSORY AND NUTRIENT PROFILE

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Abstract:

Aegle marmelos (Bael fruit) a fruit recognized for its medicinal and nutraceutical properties has its history in treating various medical conditions. The properties of the plant made it as a valuable medicinal compound in the traditional treating methods. The present study was conducted with an objective to incorporate the powdered bael fruit powder (unripe, ripe and over ripe) into the chocolate and evaluate its properties. The fruits at varying levels of maturity were procured from local markets of Coimbatore. Dehydrated bael fruit powders were incorporated into the chocolate in different proportions and evaluated for its acceptance through sensory analysis. The ideal proportion from sensory analysis was subjected to textural, nutrient and shelf-life analysis. The textural analysis showed that the chocolate needs an average force of 21.17 N to get an initial break; a total force of 516.32 N and 0.2697 Joule energy to break into two halves and had a uniformity ranging from 4 N to 1 N. The nutrient analysis of the chocolate revealed that 100 g of bael chocolate possessed 552 kcal energy; 0.75% moisture; 7 g protein; 59 g carbohydrates; 32 g fat; 1.1 g ash; 9.9% crude fiber; 5.4 mg iron. The shelf-life analysis of the chocolate carried for 1 month revealed that the product was not deteriorated in its quality.The study signifies the possibility of incorporating the under-utilized fruits into snack foods. Thus, the value addition of foods with fruits can enhance functional and nutritional value of foods.

Introduction:

*Aegle marmelos*a world wide grown fruiting tree belongs to the family *Rutaceae.* It is one of the under-utilised plants with immense medicinal properties. The disease improving capacity of the fruit has been proven with various in vitro and in vivo studies.[(1)]The objective of the present study was to incorporate the fruits at various levels of maturity into chocolate. The

formulated chocolates will be subjected to the textural, nutritional and shelf life analysis which is followed by packaging and labelling.

Methodology:

Selection of fruit

The utilization of the plants as a potential source of medicinal compounds has been dated since thousands of years. Such plants were used for the treatment of various physical and mental illness[(2)]. *Aegle marmelos* is one such plant with immense medicinal benefits which was chosen for the incorporation into the chocolates.

Preparation and Incorporation of fruit powder

Ripe fruits of *Aegle marmelos* were pulped out from the outer coat and dried at 100 C for 5-12 hours. The dehydrated pulp was powdered in a food processor. The ripe fruit powders were incorporated at varying proportion ranging from 5 – 25% into the chocolate for selection of ideal proportion.

Selection of Ideal Proportion

Sensory Evaluation was conducted to evaluate and select the ideal proportion of ripe bael fruit powder for incorporation. The chocolates were evaluated with the scale provided by Popov-Raljic, 2009 [(3)].

Textural Analysis

Texturometers or texture analyzers measure the textural characteristics of a food product. The principle of the system is to physically deform the test sample and measure the response of the sample. The responses of the product correlate specific sensory textural properties. The results of the textural properties are interpreted. The properties such as hardness cut and shear, uniformity of the chocolates was measured.

Nutrient Analysis

The formulated chocolate was analysed for its nutrient contents such as Carbohydrates (Difference method)[(4)]; Protein (Kjeldahl method)[(5,6)], Ash[(7)], Fat (Soxhlet method), Iron (Wong Method) [(8)], Crude Fiber[(9)] and Total calories (Atwater system).

Shelf-life Analysis

Shelf life of a food product is mainly determined with the moisture content. The moisture content of the formulated product was estimated with the moisture analyzer. The moisture analyzer is set with the time and temperature. Approximately 2 g of the sample is weighed and subjected to moisture analyser (Sartorius Moisture analyser). The formulated chocolate was packaged and labelled.

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Results:

Sensory evaluation of the formulated product:

Sensory evaluation is a scientific method that evokes, measures, analyzes and interprets response of products, through sense of sight, smell, touch, taste[10]. 20 panel members evaluated the formulated product and the average responses of them were tabulated in the table – 1

Table 1: Sensory evaluation of Bael chocolate

Characters	Std	Var 1	Var 2	Var 3	Var 4	Var 5
Appearance	4.95 ± 0.22	4.55 ± 0.69	4.35 ± 0.81	3.85 ± 0.99	3.45 ± 1.39	3.35 ± 1.23
Texture	4.95 ± 0.22	4.65 ± 0.49	4.25 ± 0.72	3.7 ± 0.92	3.05 ± 0.89	3.05 ± 1.19
Chewiness	4.95 ± 0.22	4.3 ± 0.73	3.95 ± 0.94	3.4 ± 0.92	3 ± 0.92	2.75 ± 1.12
Aroma	4.8 ± 0.52	4.4 ± 0.68	3.9 ± 0.85	3.45 ± 0.89	3.1 ± 1.02	2.95 ± 1.0
Taste	4.85 ± 0.37	4.1 ± 0.97	3.7 ± 0.92	3.05 ± 0.83	2.35 ± 1.09	2.35 ± 1.23
Total	**4.9 ± 0.25**	**4.4 ± 0.54**	**4.03 ± 0.70**	**3.49 ± 0.78**	**2.99 ± 0.87**	**2.86 ± 0.96**

Std - standard

Var 1 - variation 1 (5 g ripe powder + 95 g milk chocolate)

Var 2 - variation 2 (10 g ripe powder + 90 g milk chocolate)

Var 3 - variation 3 (15 g ripe powder + 85 g milk chocolate)

Var 4 - variation 4 (20 g ripe powder + 80 g milk chocolate)

Var 5 - variation 5 (25 g ripe powder + 75 g milk chocolate)

The bael fruit being rich in functional properties its incorporation in snack foods will lead to value addition. Chocolate is the most commonly consumed snack by the children, hence incorporation of the powder into the chocolate with different concentration was carried out. The sensory evaluation was done by 20 semi qualified panel members to assess the products with varying criteria.

The sensory evaluation results were consolidated and the variation which obtained high score was chosen for the formulation. The variation 1 received the highest scores after to that of the standard. Hence variation 1 i.e. 5 g of the ripe fruit powder and 95 g of the milk chocolate was considered as the apt level of incorporation.

Kadge et.al., evaluated the sensory attributes of the lime infused bael syrup. The results of the study coincides with the present study where the highest accepted variant was with the incorporation of 3g of fruit pulp.[11]

Physical analysis of the formulated product:

Texture analysis

Texture is important in determining the eating quality of foods and can have strong influence of food intake [(12)]. The characteristics such as hardness, uniformity in texture, Total Hardness, cutting shearing were analyzed using the texture analyzer and the results were expressed in the graphs as follows.

Hardness Test

Hardness may be defined as the mechanical textural attribute relating to the force required to compress the sample. The test was carried out by compressing the sample with molars and evaluating the required force [(13)]. The hardness analysis of the formulated product resulted that the chocolate needs an average force of 21.1772 N (Newton) to break. Graph was plotted for the analysis and it was shown in the figure – 1.

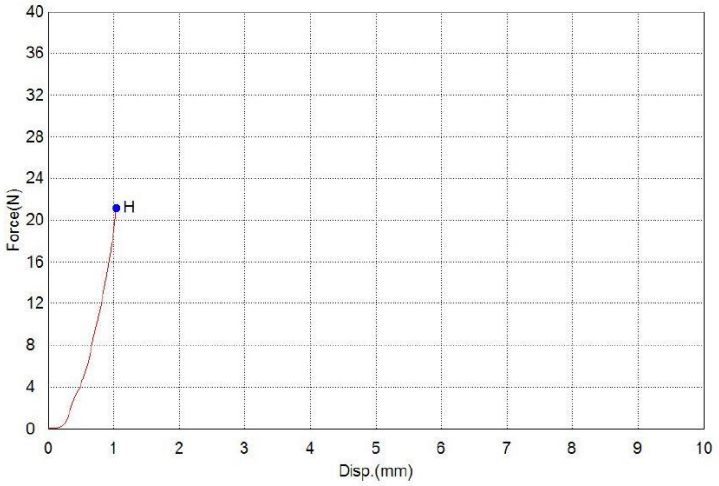

Figure 1: Hardness for Bael chocolate

Liang, (2004) studied on the change in hardness of the chocolate on using variety of milk powders. The different variation of chocolates had hardness ranging from 20 – 40 N. This fluctuation is due to the method used to dry the milk. [(14)] The present study also had the hardness within the reported study's range.

Cutting and Shear Test

Cutting and shear test is one of the commonly used methods in Food texture analysis. This calculates the total amount of force and energy required to cut the product into halves. The formulated product requires total force of 516.325 N (Newton) and 0.26977 Joule of energy to break into halves. The results of the tests are shown in the figure – 2.

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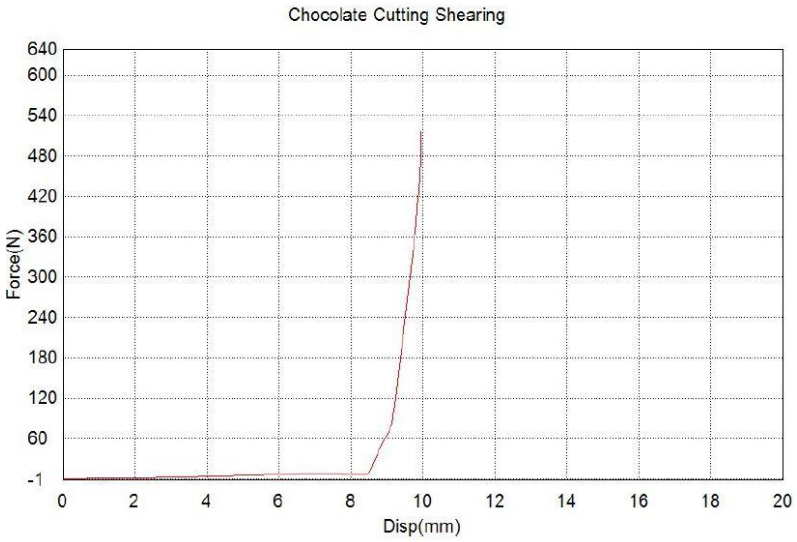

Figure 2: Cutting and Shearing test of the Bael chocolate

Uniformity test

Uniformity of the product indicates the degree to which the ingredients are evenly dispersed in the product. This includes the degree to which the chewing characteristics are even throughout the mastication. The uniformity test of the formulated product was carried out using shear and cut test. The uniformity of the product ranged from upper peak value of 4 Newton to lower peak value of 1 Newton which denoted the slight deviation from uniform texture. The total area hardness of the product was 73.5451 Newton. The graph plotted for the uniformity was shown in figure – 3.

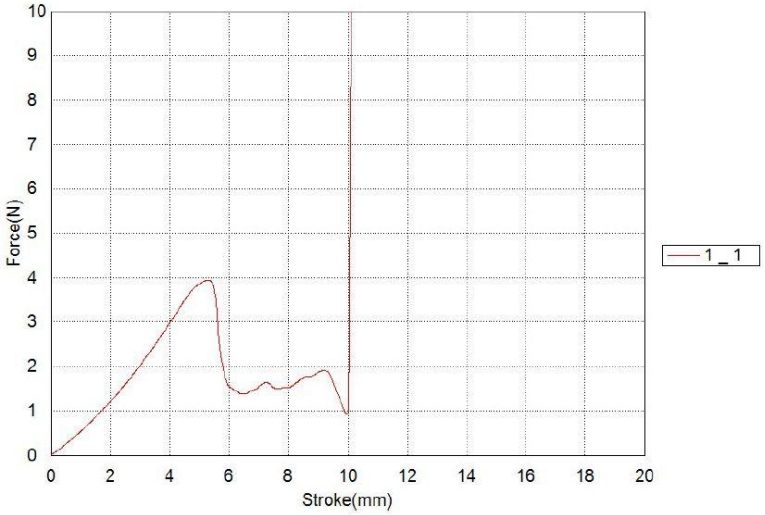

Figure 3: Uniformity test of Bael chocolate

Nutrient Analysis of the formulated product:

Analysis of the nutrients refers to the determination of the major components of the foods such as Moisture, Ash, Fat, Protein, Carbohydrates, Energy, Crude fiber and Iron [(15)]. The nutrient analysis of the formulated chocolate was carried out. The results are interpreted in the table – 2

Table 2: Nutrient composition of the Bael chocolate

Nutrient with unit	Standard	Variation 1
Energy (kcal)	570	552
Moisture (%)	0.9	0.75
Protein (g)	7	7
Carbohydrates (g)	59	59
Fat (g)	34	32
Ash (g)	0	1.1
Crude fiber (%)	0	9.9
Iron (mg)	0	5.4

The formulated chocolate was analysed for the basic nutrients to be tested for chocolate. The incorporation of the fruit powder had an influence on the nutritive value of the product. The iron, total mineral content and fibre content was increased on incorporation of the fruit powder to the chocolate.

Shelf life analysis:

Moisture is an important factor in food quality, preservation and resistance to deterioration [(15)]. The effect of moisture on the stability and quality of foods makes it of greater importance to consider to prolong the shelf life of the product[(16)]. The moisture content of the formulated chocolate was analyzed at a time interval of 1 week for the period of one month. The results are provided in the table – 3

Table 3: Moisture analysis of the Bael chocolate

Time interval	Moisture content
1st week	0.75
2nd week	0.91
3rd week	6.8
4th week	2.28

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The moisture content of the formulated chocolate was less than 1 percent at the beginning of the study. The moisture gradually increased on further storage and reduced to 2.28% during the 4th week. This may be due to the drying of the chocolates on the storage that reduces its moisture content. This reduction in moisture can be controlled by storing the chocolate in proper refrigerator conditions. The moisture loss affects only the appearance of the product but does not affect the quality of the product.

Packaging and labelling:

The formulated chocolate is primarily packed in aluminium foil. The packed chocolate is further covered with external wrapper as a secondary package material. The nutritional information obtained from the analysis and the ingredient lists are printed in the labels. The labelling was done as per the regulations.

Conclusion

Aegle marmelos, a fruit with potential therapeutic properties was evaluated for its acceptance. Ripe fruit powder was incorporated into the chocolates at different concentration and best variation was obtained through sensory evaluation. The formulated chocolate was evaluated for the textural and nutrient analysis along with the shelf life studies. The final end product was packaged and labelled according to the standards. Thus, value addition of snack foods with such fruits can enhance the nutritional and functional value of foods, thereby can make a positive impact on the health of the consumer.

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DEVELOPMENT AND SENSORY ACCEPTABILITY OF VALUE ADDED PRODUCTS PREPARED FROM BAMBOO RICE

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Abstract:

India is one of the largest bamboo producing countries like other tropical countries and all type of climate is also suitable for growing bamboos, while some bamboo were planted others may be grown naturally by seed dispersal and these trees yield seeds or rice once in its life time. The author here describes the value addition using bamboo rice. Bamboo rice are collected from Tamil Nadu and Kerala and attempts were made to prepare using bamboo rice subjected to different pre-treatment and the following different products prepared by allotted codes namely Pasta (BRPP), Sweet Cookies (BRSWC), Spicy Cookies (BRSPC) and Gulab Jamun (BRGJ).These products are also standardized by using 5 point hedonic scales.10 trained panel members evaluated the products during sensory evaluation. From the result the present study shows the product BRGJ shows good quality, sensory and consumer acceptability.

Keywords: Bamboo Rice, Pasta, Sweet Cookies, Spicy Cookies, Gulab Jamun, Sensory Evaluation.

Introduction:

There are over 1,250 woody bamboos in the world in approximately 75 genera. They are native to Africa, the Americans, Asia and Oceania and have been introduced to Europe (Liese and Kohl, 2015). Bamboo rice has become an important and major source of income for tribal living in the forest (Siyanna, 2020). Bamboo rice is special rice that is grown out of a dying bamboo shoot. When the bamboo shoot breathes its last, it flowers in to a rare variety of

rice seeds, which are known as bamboo rice (Sarika Rana, 2017). In bamboo, the fruit is one seeded structure that does not split when ripe (Wong, 2004).

Bamboo rice is also known as Mulayri in Malayalam Language and Moongil Arisi in Tamil Language by the tribal of southern India. This rice is rich in carbohydrates, proteins, amino acids, fibre, vitamins and minerals (Shillpi Singh, 2021). Bamboo seeds are nutrient rich and replace paddy in many tribal villages of Western Ghats and the North East. In fact protein content of bamboo seed is higher than that of rice and wheat. Other than protein the rice also has carbohydrates, vitamins including A, B_1, B_2, B_3, B_6, minerals like calcium, iron, phosphorus, and fibre. This makes the rice highly nutritive especially to small children because of the immediate release of energy it gives (Meenakshi Bharathi, 2019).

There is a huge market for products prepared from the bamboo shoots. The season for shoot procurement is between June and September. We process the shoots for preparations of pickles and such other items (Revathy, 2014)**.** Farmers are facing several problems, including low yield due to adverse weather and natural calamities, besides poor prices. Converting their produce in to value-added products is the only way to help them. It will also save them from getting duped by middle men. Bakery products are made using bamboo rice and shoots, millets, wheat and other organically-grown cereals and vegetables (Kuruvila, 2019).

In the light of the above facts realizing the importance of bamboo rice the study were undertaken with the following objectives

1. To prepare pasta from bamboo rice
2. To prepare sweet cookies from bamboo rice
3. To prepare spicy cookies from bamboo rice
4. To prepare gulab jamun from bamboo rice
5. To assess the sensory evaluation of the prepared product
6. To know the shelf life, storage quality of the prepared product under room temperature
7. To know the shelf life, storage quality of the prepared product under refrigerated temperature

Methodology:

The research work was carried out in the Department of Nutrition and Dietetics, Sadakathullah Appa College (Autonomous) Rahmath Nagar, Tirunelveli Tamil Nadu, during the academic year from December 2018- April 2019.

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Selection of raw material:

The main raw material (bamboo rice) needed for the study was collected from Wayand (Kerala) and Tirunelveli (Tamil Nadu). The samples has been collected as per the requirement and stored in refrigeration for future uses. It was collected during the month of December 2018.

Figure 1: Bamboo Rice

Figure 2: Powdered Bamboo Rice

Identification of the sample

The bamboo rice was identified with the help of a Dr. Cissie Theeblyn David, Assistant Professor, Department of Food Science and Nutrition ICAR-KVK, Tirupathisaram, Kanniyakumari District, Tamil Nadu, India.

Selection of other ingredients

The Ingredients like wheat flour, butter, sugar, khoa, curd, ajwain, salt, cardamom, ghee, green chilli, coriander leaves, and ginger were purchased from a local super market near Tirunelveli and stored for research purpose.

Pretreatment employed

The samples bamboo rice after collecting was cleaned by removing the stones and dust particles. The bamboo rice was thoroughly washed in running water 2-3 times. Then it was shade dried, and with careful attention the samples were packed in polyethylene bags and stored in refrigeration for further analysis as per Prasad *et al.* (2020)

Preparation of bamboo rice powder

Bamboo rice once procured from shop was sorted, cleaned in running water for 2 to 3 times, sun dried and powdered in a mixer for preparing the product

Material used for the study:

Electronic weighing balance

Electronic weighing balance of AY-220 model was used to weigh the samples in terms of grams and milligrams.

Refrigerator

Refrigerator (Kelvinator) with a temperature range of 6-20^{0}C was used to store the samples.

Mixer

Mixer model of 2k-458 was used to grind the samples for separation.

Baking Oven

Baking oven of model HD6975/00 was used to bake the sweet and spicy cookies.

Strainer

Stainless steel strainer were used for the extraction of the sample

Other Minor Equipment

Other materials like stainless steel cups, mugs, plates, knives, steel tray, baking trays, roller, and cutter were used for the preparation of the product.

Formulation of the product:

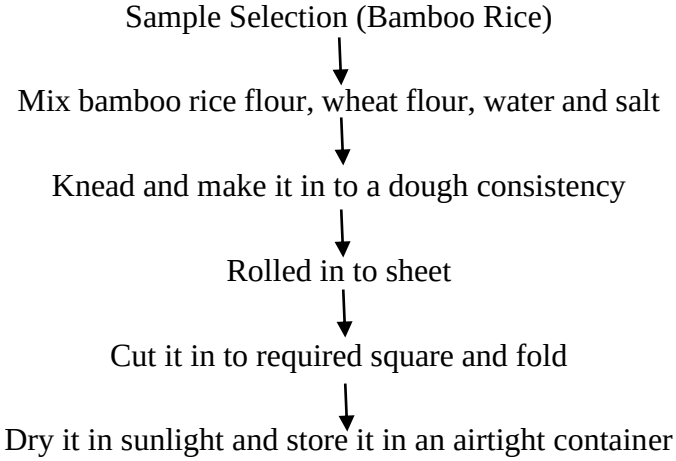
Preparation of Pasta (BRPP)

Table 1: Ingredients used for making pasta

Sr. No.	Ingredients used	Amount
1	Bamboo Rice	100 g
2	Wheat Flour	50 g
3	Salt	02 g
4	Water	25 ml

Pasta was prepared from 100 g of bamboo rice, 50 g of wheat flour, 02 g of salt and 25 ml of water. Methodology for preparing pasta from bamboo rice is given below in flow chart 1.

Flow Chart 1: Methodology for Preparing Pasta (BRPP)



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Preparation of Sweet Cookies (BRSWC)

Table 2: Ingredients used for making sweet cookies

Sr. No.	Ingredients used	Amount
1	Bamboo Rice	100 g
2	Sugar	75 g
3	Butter	50 g

Sweet cookies were prepared from 100 g of bamboo rice, 75 g of sugar, and 50 g of butter. Methodology for preparing sweet cookies from bamboo rice is given below in flow chart No: 2

Flow Chart 2: Methodology for Preparing Sweet Cookies (BRSWC)

Samples Selection (Bamboo Rice)

↓

Butter is creamed well

↓

Mix bamboo rice flour, butter and sugar

↓

Knead and make it in to a dough consistency

↓

Dough is rolled in to 1/4th thick sheet

↓

Cut it in to round shape and Place on greased tray

↓

Bake with the help of oven

↓

Bake with the temperature of 250^{o} C for 15-20 minutes

Figure 3: Pasta made from Bamboo Rice

Figure 4: Sweet Cookies made from Bamboo Rice

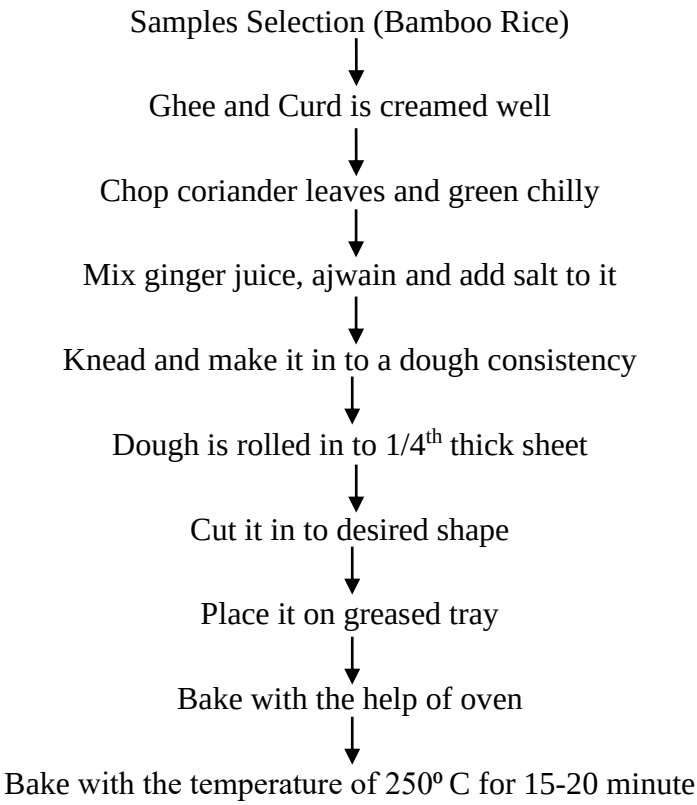
Preparation of Spicy Cookies (BRSPC)

Table 3: Ingredients used for making spicy cookies

Sr. No.	Ingredients used	Amount
1	Bamboo Rice	100 g
2	Curd	20 ml
3	Salt	02 g
4	Ajwain	05 g
5	Green Chilly	1 No
6	Coriander leaves	05 g
7	Ginger	05 g
8	Ghee	10 g

Cookies were prepared from 100 g of Bamboo rice flour, 20 ml of curd, 02 g of salt, 05 g of ajwain, green chilly 1 no, 05 g of coriander leaves, 05 g of ginger and 10 g of ghee, Methodology for preparing spicy cookies from bamboo rice is given below in flow chart no: 3

Flow Chart 3: Methodology for Preparing Spicy Cookies (BRSPC)



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Preparation of Gulab Jamun (BRGJ)

Table 4: Ingredients used for making gulab jamun

Sr. No.	Ingredients used	Amount
1	Bamboo rice	75 g
2	Sugar	100 g
3	Khoa	50 g
4	Cardamom	05 g
5	Water	50 ml

Gulab Jamun was prepared from 75 g of bamboo rice, 100 g of sugar, 50 g of khoa, 05 g of cardamom and 50 ml of water. Methodology for preparing gulab jamun from bamboo rice is given in the below flow chart no: 4

Flow Chart 4: Methodology for Preparing Gulab Jamun (BRGJ)

Dissolve sugar in water Add cardamom to it

↓

Bring it to boil

↓

When sugar solution gets thread stage, remove it from stove

↓

Keep it aside and cool

↓

Khoa is smoothly kneaded and bamboo rice flour is mixed with it

↓

Bring it to ball shape of required size

↓

Heat ghee and fry the prepared balls in it till it becomes golden brown

↓

Then soak it in the sugar syrup

Figure 5: Spicy Cookies made from Bamboo Rice

Figure 6: Gulab Jamun made from Bamboo Rice

Sensory evaluation

Sensory Evaluation of the Prepared Pasta

The prepared products were subjected to 10 trained panel members for a point five hedonic rating scale and results were recorded.

Figure 7: Sensory Evaluation of the Prepared Cookies and Gulab Jamun

Figure 8: Sensory Evaluation of the Prepared Pasta

Shelf Life Study

Each and every product must possess a good shelf life study. For this the prepared products were carefully observed in room temperature storage and in refrigerated storage for about 21 days.

Results and Discussions:

Sensory Evaluation of the Prepared Product

The formulated product was organoleptically evaluated by 10 trained panel members. The panel members were asked to evaluate the product for appearance, texture, taste, color, flavor, and overall acceptability. The mean score obtained for the formulated product is given below in fig no 09 to fig no 12

Sensory Evaluation of the Prepared Pasta

With reference to fig no 09 the product prepared pasta scored the highest value in appearance 4.6 and the lowest goes to flavor 3.7, colour 4.4, taste 4.1 followed by texture 3.9 and with an overall acceptability of 4.3

Sensory Evaluation of the Prepared Sweet Cookies

Regarding fig no 10 sweet cookies values obtained are for appearance 4.2, colour 3.9, flavor 4.2 taste 4.3, texture 4.1 and with an overall acceptability of 4.5

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Sensory Evaluation of the Prepared Spicy Cookies

With respect to fig no 11, Spicy cookies shows the lowest score to colour 3.9 and the highest goes to appearance 4.8, followed by texture 4.0, flavor 4.2, overall acceptability of 4.4 and taste 4.7

Sensory Evaluation of the prepared Gulab Jamun

Gulab Jamun fig no 11, exhibits the lowest score to flavour 4.2 and the highest goes to appearance 4.8, followed by colour 4.6, texture 4.4, and taste 4.7 and with an overall acceptability of 4.5.

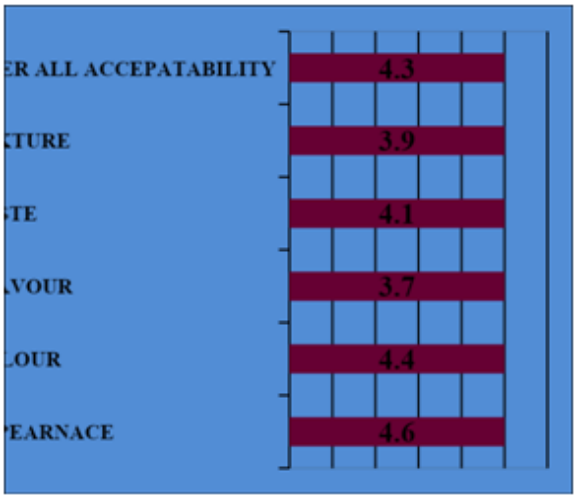

Figure 9: Sensory Evaluation of the Pasta

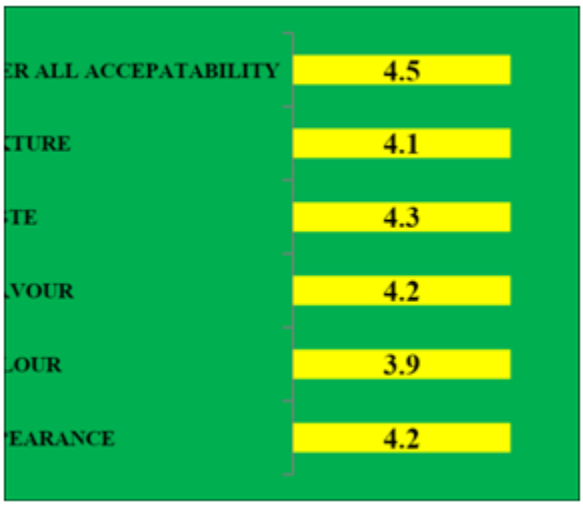

Figure 10: Sensory Evaluation of the Sweet Cookies

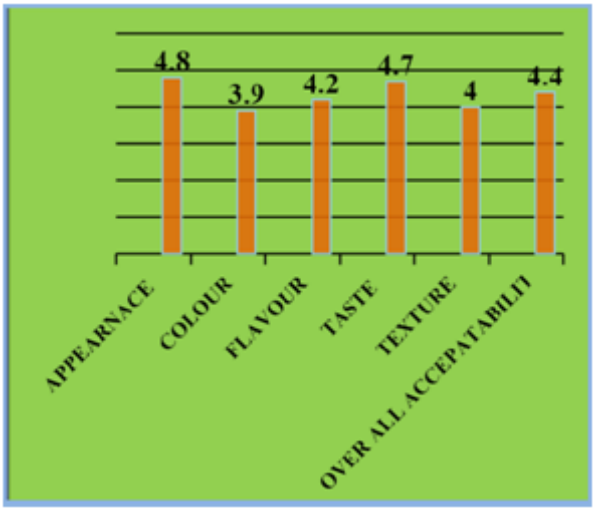

Figure 11: Sensory Evaluation of the Spicy Cookies

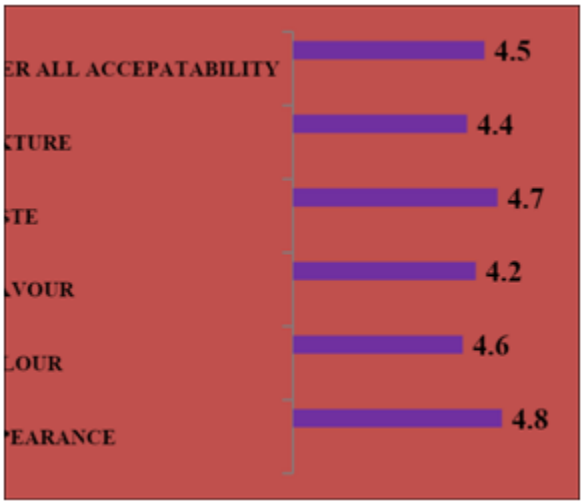

Figure 12: Sensory Evaluation of the Gulab Jamun

Storage study and shelf life of the prepared product

The selected products were stored in airtight plastic containers till analyzing various parameters. Care was taken to see that they were stored in clean, dry place and away from sunlight and pests. Prepared products were stored in normal room temperature and in refrigerated temperature to assess their storage pattern. Every day the containers were checked to see any microbial growth and the development of off flavor.

Table 5: Shelf life of the prepared products under room temperature

Sr. No.	Days	Room Temperature Storage			
		Pasta (BRPP)	Spicy Cookies (BRSWC)	Sweet Cookies (BRSPC)	Gulab Jamun (BRGJ)
1	1-7th	No Change	No Change	No Change	No Change
2	8-14th	No Change	Change in Colour	Change in Colour	No Change
3	15- 21st	Colour Change	Slightly Off Flavour	Slightly Off Flavour	No Change

Table 5 reveals the shelf life of the prepared products under room temperature. The prepared product pasta showed no changes till second week but colour change is observed in the third week. Regarding spicy cookies and sweet cookies no changes were viewed till first week but colour change is observed in the second week followed by slightly off flavor developed in the third week. For the product gulab jamun no changes were observed till the third week under room temperature storage.

Table 6: Shelf life of the prepared products under refrigeration temperature

Sr. No.	Days	Room Temperature Storage			
		Pasta (BRPP)	Spicy Cookies (BRSWC)	Sweet Cookies (BRSPC)	Gulab Jamun (BRGJ)
1	1-7th	No Change	No Change	No Change	No Change
2	8-14th	No Change	No Change	No Change	No Change
3	15- 21st	No Change	Change in Colour	Change in Colour	No Change

Table 6 reveals the shelf life of the prepared products under refrigeration temperature. The prepared product pasta and gulab jamun showed no changes till third week. Among the

products analysed for spicy cookies and sweet cookies no changes are seen till second week but colour change is observed in the third week under refrigeration temperature storage.

Conclusion:

It was concluded that there are limited studies in value addition in bamboo rice. Bamboo rice is a source of natural product and so a trial was made formulating using the same. As we all know that all the products we get from shops are fully loaded with lots of preservatives beyond the permissible level. When we move on these types of naturally based preparation surely it will have a positive effect on health as well as mind too. Even people from 6 to 60 years can use these types of foods which do not cause any side effect.

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BIOSURFACTANTS: PROPERTIES AND STRUCTURAL CHARACTERIZATION

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Abstract:

Biosurfactants (BS's) are amphiphilic molecules with hydrophilic and hydrophobic moieties which facilitate their easy diffusion and hence have various applications in different fields. BS's reveal several properties like biodegradability, lower toxicity, ecological acceptability, bioavailability, specificity and ability to be produced from renewable and cheaper substrates. Collectively due to diverse properties and structural diversity shown by BS's they are considered as a potential group for various industrial, environmental, pharmaceutical and biotechnological applications. Since, production of BS is reported from different microorganisms like bacteria, fungi and actinomycetes. Based on the source of BS, there is variation in their structure and based on their functional groups, different BS are reported for their various applications. Hence, structural characterization is an important aspect for specific application of any BS. This review aims to compile information on properties of BS and their structural characterization.

Keywords: Biosurfactant, Properties, Structural characterization, TLC, NMR.

Introduction:

Surfactants derived from microorganisms are known as 'Biosurfactants'. They show colossal diversity in terms of structure and performance chemically they are amphipathic molecules that lower the physical phenomenon at interface consequently increasing the solubility of water immiscible substances. They are made up of hydrophobic and hydrophilic moieties (Ghasemi *et al.*, 2019). Hydrophobic 'tail' consists of hydrocarbon chain of saturated/unsaturated and hydroxylated fatty alcohols and the hydrophilic 'head' is comprised of a polar group that contains mono, oligo or polysaccharides and peptides (Nayarisseri *et al.*, 2019) (Fig.1). The

apolar moiety is usually a hydrocarbon chain, whereas polar moiety is ionic (cationic or anionic), non-ionic or amphoteric (Paraszkiewicz *et al.*, 2019).

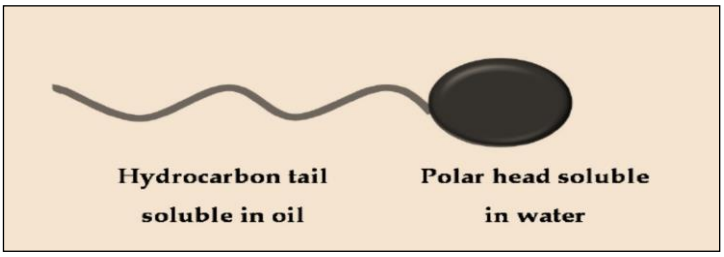

Figure 1: Surfactant molecule with non-polar head and polar tail

BS enhances the solubility of hydrophilic molecule and successively, causes the reduction in surface and interfacial tension at oil/water interface. The critical micelle concentration (CMC) is that, the concentration of surfactant at which organized molecule assembles to create micelles which correspond to the arrangement at which tensioactive agent achieves lowest stable surface tension. BS's are produced under various growth and environmental conditions and are reported to be mainly involved in increasing the solubility and availability of assorted water immiscible substrates. BS's exhibit several advantages over their chemical counterparts in terms of lower toxicity, greater biodegradability and specificity. Their ability to function during a wide selection of utmost conditions, and also their biocompatibility and digestibility make them excellent candidates to be used in agriculture, food industry (Nitschke and Silva, 2018), soil and water remediation (Joshi *et al.*, 2013;Mulligan, 2017), microbial enhanced oil recovery (Nayaresseri *et al.*, 2019), biomedical science (Joshi *et al.*, 2013), nanotechnology (Singh *et al.*, 2017) and other diverse fields including usage in detergent and cleaning solutions (Kourmentza *et al.*, 2017). BS's are active at very low concentrations and stable at extreme conditions of pH, salinity and temperature (Joy *et al.*, 2017) at low critical micelles concentration (CMC) (Fig. 2).

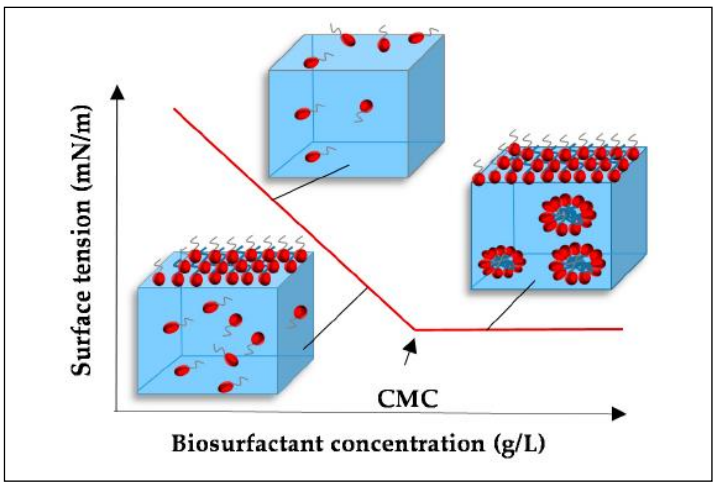

Figure 2: Regions showing micelle formation (CMC) Properties of biosurfactants

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Thanks to such unique functional properties and eco-friendly nature, these biological surfactants are expected to become multi-functional constituents of the twenty-first century. Additionally, they have additional applications in industrial sectors like pharmaceutical, textile processing, agricultural, cosmetics, personal care, food industries, and environmental applications like hydrocarbon degradation, soil remediation and oil recovery (Joshi *et al.,* 2013; Mulligen 2017).

Properties of Biosurfactants:

The properties of BS in comparison to chemical surfactants are broad substrate availability which makes them suitable for commercial applications. Microbial surfactants are identified with their surface movement, resilience to pH, temperature and ionic quality, biodegradability, low poisonous quality, emulsifying and demulsifying capacity and antimicrobial action (Sansarode and Sahasrabudhe, 2018).

- **Surface and interface activity**

BS helps in decreasing surface strain and the interfacial pressure. Surfactin produced by *B. subtilis* is known to cut back the surface tension of water to 25mN/m and interfacial strain water/hexadecane to under 1mN/m (Sansarode and Sahasrabudhe, 2018). *P. aeruginosa* produces rhamnolipids which diminished surface tension of water to 26mN/m and interfacial strain of water/hexadecane to esteem under 1mN/m (Chakrabarti, 2012). BS's are more powerful and effective and their CMC is around few folds below chemical surfactants, i.e for maximal decline on surface strain, less surfactant is the key.

- **Temperature and pH tolerance**

BS production from extremophilic microorganisms has gain consideration within the last decade for their commercial application. A huge portion of BS's and their surface action are safe towards natural factors, as an example, temperature and pH. Noha *et al.,* (2005) reported that lichenysin from *Bacillus licheniformis* is challenging to temperature up to 50°C, pH within the vicinity of 4.5 and 9.0 and NaCl and Ca concentration up to 50 and 25g/l. Another BS produced by *Arthrobacter protophormiae* was reported to be stable at broad range of both temperature (30-100°C) and pH (2 to 12) (Sansarode and Sahasrabudhe, 2018).

- **Specificity**

Microbially produced BS's are generally very specific with their substrates. Other than this, a selected sort of BS is produced by specific microorganisms only eg, surfactin is produced

by *Bacillus subtilis* and rhamnolipids are produced by *Pseudomonas sp* (Sansarode and Sahasrabudhe, 2018).

- **Biodegradability**

BS's produced by microorganisms are easily degraded as compared to synthetic surfactants and hence considered appropriate for natural applications like bioremediation/biosorption. The day by day increase in ecological concern compelled us to seem for elective items rather than chemical agents, for instance, biosurfactants, enzymes, etc. BS's from marine microorganisms were concerned for the biosorption of ineffectively solvent polycyclic sweet smelling hydrocarbon, phenanthrene contaminated in aquatic surfaces. The blossom of marine algae cochlodinium was controlled by utilizing the biodegradable BS sophorolipid with the removal efficiency of 90% for each 30 min treatment (Gharaei-Fathabad, 2011).

- **Low toxicity**

BS's are considered to be non-harmful and are proper for pharmaceutical, corrective and sustenance employments. It had been observed that the LC50 of the chemical-derived surfactant against *Photobacterium phosphoreum* was found to be 10 times on top of rhamnolipids. The low toxicity profile of BS like sophorolipids from *Candida bombicola* made them helpful in nourishment ventures (Sansarode and Sahasrabudhe, 2018).

- **Emulsion framing and emulsion breaking**

Emulsions are often depicted as a heterogeneous framework, comprising of one immiscible fluid scattered in another as beads, whose distance across by and huge surpasses 0.1mm. Emulsions are of two types: oil-in-water or water-in-oil emulsions. They need minimal stability which might be balanced out by adding substances like BS. BS's can sustain as steady emulsions for a substantial length of time to years.

- **Antiadhesive agents**

A biofilm is a group of microbes/other organic matter that have aggregated on any surface. The primary step in biofilm foundation is bacterial adherence over the surface was influenced by different components including type of microorganism, hydrophobicity and electrical charges of surface, ecological conditions and capacity of microorganisms to deliver extracellular polymers that assists cells to stick to surfaces (Jadhav *et al.,* 2011). BS's are able to be utilized for changing the hydrophobicity of the surface which as a consequence influences the bond of microorganisms over the surface. A BS from *Streptococcus thermophilus* backs off the colonization of other thermophilic strains of Streptococcus over the steel which are accountable

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of fouling also a BS from *Pseudomonas fluorescens* slowed down the attachment of *Listeria monocytogenes* onto steel surface (Konishi *et al.,* 2008).

Structural characterization of biosurfactant:

The chemical structure and behavior of BS's vary from one microorganism to a different. In fact, a microorganism may simultaneously synthesize a combination of BS's by distinct molecular configurations (Rodrigues *et al.,* 2006). Based on that, it also affects its stability at various pH, temperature and salinity conditions. Indubitably, it's the structure of the BS molecule that determine it's behavior as a powerful emulsifier or a proper surfactant each of which has its special applications in the industrial and environmental issues. Moreover, a finding of the chemical structure of BS can shed light on the mechanisms of their action in numerous applications and in designing more optimized processes. There are chromatographic and spectroscopic methods applied to characterize BS. Among the foremost employed techniques are Critical micelle concentration (CMC) thin layer chromatography (TLC), Nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FT-IR), high-performance liquid chromatography (HPLC) and Mass spectrometry (ESI-MS/MS^2) (Fenibo *et al.,* 2019).

- **Critical Micelle Concentration (CMC)**

The CMC value of any BS is an indicator of its surfactant potential hence a strong surfactant must have a lesser CMC value. Anvari *et al.,* (2015) reported CMC of BS produced by *Bacillus tequilencis* is 0.12g/l while Ismaeil *et al.,* (2013) have reported CMC of 200mg/l for BS produced by Bacillus sp 1-15. These results were in agreement with Phulpoto *et al.,* (2020) who have reported CMC of 40mg/l from *Bacillus nealsonii* S2MT.

- **Thin Layer Chromatography (TLC)**

Initial characterization of the BS is evaluated using TLC. It is a technique used to separate non-volatile mixtures, to monitor the progress of a reaction, identify compounds present in a given mixture and determine the purity of a substance. Khademolhosseini *et al.,* (2019) separated a fraction of the crude BS on a silica gel plate using $CHCl_3$: CH_3OH : H_2O (70 : 10 : 0.5, v/v/v) as the developing solvent system with a color developing reagents (iodine and anthrone). Jemil *et al.,* (2017) and Al-Waihibi *et al.,* (2016) have confirmed lipopeptide nature of BS produced by *Bacillus methylotrophicus* DSC1and *Bacillus subtilis* strain BIA respectively. Also, lipopeptide of Bacillus sp (SB2) was confirmed by TLC (Joy *et al.,* 2017). Ramybharti *et al.,* (2018) have assured the presence of surfactin and iturin from *Bacillus subtilis* Bbv57 by TLC.

- **Fourier transform infrared spectroscopy (FT-IR)**

FTIR is the most useful analysis for identifying types of chemical bonds (functional groups) present in the biologically active fraction of an unknown BS and thus determines its chemical nature. In a study done by Elazzazy *et al.,* (2015) the FT-IR spectrum showed extending vibration at 3300– 3500 cm^{-1} which is characteristic of N–H stretching vibrations, indicating strong hydrogen bonding. The strong band peak at 3000–2900 cm^{-1}, was characteristic of aliphatic chains' ($–CH_3$, $–CH_2$) stretching vibrations. The manifestation of a weak absorbance signal at 2300–2400cm^{-1} may be due to C-N stretch, The bands observed at 1650cm^{-1} was a particular indicator of linkages between the amides I and II. The absorbance in this region was important in the presence of the peptide group in the molecule. High intensity peak in the region of 1000–1100cm^{-1} was assigned to O–C–O extend vibrations of carboxylic acids, aldehydes and ketones. It is remarkable, representing the oxidation of the hydroxyl groups of hydrolysates (originated from the medium peptides). This FT-IR profile of the BS showed similarity to surfactin, a lipopeptide BS and other lipopeptide BS's like arthrofactin and lichenysin (Lin *et al.,* 1993) confirming the lipopeptide nature of a BS. In another study by Chandankere *et al.,* (2013) an efficient BS of *Bacillus methylotrophicus* USTBa was isolated from petroleum reservoir was characterized by FT-IR spectrum. Characterization of the BS by FTIR was also performed by Zhou *et al.,* (2015) of *Bacillus sp*. ZG0427. Similarly, Joshi *et al.,* (2016) also used FTIR analysis for characterization of crude BS in the range of 400 and 4000 wave numbers (cm^{-1}).

- **Nuclear Magnetic Resonance (NMR) Analysis**

NMR spectroscopy is a scientific method that provides the structural information of molecules in solution with a high resolution. NMR spectroscopy is a high throughput technique. Structure determination by NMR is also divided into the subsequent steps: (1) Establishing suitable conditions for recording spectra; (2) Measuring a series of 1D (^1H and ^{13}C) or 2D (e.g. TOCSY, COZY and ROSY) NMR spectra; (3) Integrating cross peaks and transformation into upper-distance bounds (calibration); (4) Assess the caliber of the molecular structure. NMR can be used to confirm lipopolysacharide structural identification of both the peptide and fatty acid portion while simultaneously providing data on the position of linkages between the peptide and fatty acid chain. For lipopolysacharide NMR experiments, purified lipopolysacharide is dissolved in deuterated chloroform, and a series of 1D and 2D NMR experiments are carried out. Results from NMR spectroscopy are drawn from the NMR spectrum which depends on the effect of shielding by electrons orbiting the nucleus. Chemical shifts within the spectrum symbolize alterations or changes in the molecular structure. The chemical shift for ^1H NMR is set because the difference (in ppm) between the resonance frequency of the observed proton and a reference

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proton present in a reference compound set at 0ppm. All 1D and 2D ^{1}H NMR spectra of lipopolysacharide produced by *B. licheniformis* were recorded at 299K locked to the deuterium resonance of the solvent, DMSO-d6 (Taira *et al.*, 2017). The NMR data indicate that the peptide moiety contained seven amino acids per molecule. Complete amino acid spin systems and amino acid sequence were identified from a 2D ^{1}H phase. NMR spectra indicated the presence of a extended chain fatty acid, which contained a b-hydroxyl group. Joshi *et al.,* (2016) performed NMR analysis. The proton (^{1}H) NMR and proton decoupled ^{13}C NMR experiments for characterization of BS produced by *Bacillus licheniformis* W16. Characterization of *Pseudomonas aeruginosa* HAK01 produced BS was done by ^{13}C NMR analysis and the results indicated the presence of rhamnolipid consisted of RL1 (mono rhamnolipid) and RL2 (di rhamnolipid), which are two major types of rhamnolipids (Khademolhosseini *et al.,* 2019).

- **High Performance Thin Layer Chromatography–Electrospray Ionization Mass Spectroscopy (HPTLC–ESI–MS)**

HPTLC is one amongest the sophisticated instrumental techniques supported by the full capabilities of thin layer chromatography. Also, MS/MS technique provides information about the component structures, revealing the presence of important bioactive components. BS is separated in a completely automated HPTLC system (CAMAG, Switzerland), extracted by TLC–MS interface and analyzed directly by ESI-MS. The TLC or HPTLC are reported as quite useful tools for initial qualitative or quantitative analysis of different varieties of BS's. Joshi *et al.* (2016) separated *Bacillus licheniformis* W16 BS by TLC–MS interface under positive and negative modes. Total five major bands were scrapped, eluted and analyzed by ESI–MS.

Conclusion:

Based on the study, it is concluded that the BS shows several properties like biodegradability, lower toxicity, ecological acceptability, bioavailability, specificity and ability to be produced from renewable and cheaper substrates which makes them more suitable candidates for biological applications. In addition to this, the structural diversity which results in diverse functional properties makes them an attractive group of compounds for potential use in wide variety of industrial, environmental and biotechnological applications.

Conflict of interest:

Authors declare that there is no conflict of interest among the authors.

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FORMULATION AND EVALUATION OF POLYHERBAL SHAMPOO

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Abstract:

Herbal shampoo is utilizing since ancient period, hair is one of the important part of human body. Past few decades' people believing and easy usage chemical shampoo is a better source. Synthetic shampoo cause side effects like hair colour, texture, skin or eye irritation. *Hibiscus rosa – sinensis* L, *Trigonella foenum – graecum* L, *Lawsonia inermis* L, *Murraya koenigii* L, *Aloe barbedensis* L, *Acacia concinna* (Willd.) DC., *Sapindus mukorassi* Gaetrn., *Rosa species* L of different species used to prepare polyherbal shampoo. This shampoo not only good for hair, it is also an Eco- friendly product.

Keywords: Herbal shampoo, formulation, evaluation.

Introduction:

Plants are used for cleaning, maintaining and one of the stylish purposes to expose in world. Hair is associated with important part of a human. Keratin is one of the major protein substances present in our hair. Due to modernization and civilization people are utilizing synthetic products without any awareness and easy usage. This chemical dominating condition leads to spoil hair growth, scalp, hair condition and nutrition content, etc., Now a days people again turn back to natural products usage (Dubey *et al.*, 2004).

Herbal Shampoo is healthier to scalp and hair growth. This shampoo cleanses the surface, hair and it is eco- friendly. Natural ingredients create pleasant smell and few herbal foam producing nuts also included, without any cause to skin (Jacob *et al.*, 2015). Chemical ingredients are avoided and naturally prepared shampoos are analysed through various tests before selling in the market (Vijayalakshmi *et al.*, 2018).

This present study reveals that natural shampoo with different ingredients such as *Hibiscus rosa – sinensis* L, *Trigonella foenum – graecum* L, *Lawsonia inermis* L, *Murraya*

koenigii L, *Aloe barbedensis*L, *Acacia concinna* (Willd.)DC., *Sapindus mukorassi* Gaetrn., *Rosa species* L., are grinded as paste and make as liquid shampoo.

Materials and Methods:

Plants used:

a. *Hibiscus rosa – sinensis* L
b. *Trigonella foenum – graecum* L
c. *Lawsonia inermis* L
d. *Murraya koenigii* L
e. Aloe *barbedensis* L gel
f. *Acacia concinna* (Willd.)DC
g. *Sapindus mukorassi* Gaetrn
h. *Rosa species* L

Methodology:

Selected herbs grown in our residing area were collected and rinsed with water. *Trigonella foenum – graecum* L, *Acacia concinna* (Willd.)DC and *Sapindus mukorassi* Gaetrn are 4-5 hours soaked in the water and *Hibiscus rosa – sinensis* L leaves and flowers, *Lawsonia inermis* L, *Murraya koenigii* L leaves are grinded and *Aloe barbedensis* L gel, *Rosa species* L rose water added. All the ingredients are mixed and filtered as liquid form. Herbal shampoo made hair soft, silky and shiny.

Evaluation of herbal shampoo:

The following tests were conducted for analyse the quality of shampoo:

1. **Physical appearance test:**

The formulation is assessed through clarity, colour, odour , froth content (Bhagawat, 2020)

2. **pH content:**

The pH was determined by 10% prepared material mixed in water and noticed at normal climate 25^0 (Sharma *et al.,* 2013).

3. **Wetting:**

Wetting time was calculated noticed by using Canvas paper to sink completely. A Canvas paper weighing 0.44g was made into piece circumference measuring 1 inch. In shampoo solution Canvas paper was immersed tested by using stopwatch (Malpani *et al.,* 2020).

4. **Foaming stability:**

The prepared shampoo was spread to hair and scalp, then wash the hair. The result was analyse by manually (Valavi *et al.,* 2017).

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5. Dirt dispersion:

2 droplets of shampoo added with 10ml of deionized water. 1 droplet of India ink was added. The test tube was stoppered and shakes it 10 times. The amount of ink in the foam was estimated as None, Light, Moderate or Heavy (Sharma *et al.*, 2011).

Table 1: Herbal shampoo ingredients

Sr. No.	Botanical Name	Vernacular name	Illustration of herbs
1.	*Hibiscus rosa – sinensis* L	Chemparuthi	
2.	*Trigonella foenum – graecum* L	Venthayam	
3.	*Lawsonia inermis* L	Maruthani	
4.	*Murraya koenigii* L	Karuveppilla	
5.	*Aloe barbedensis* L gel	Katralai	
6.	*Acacia concinna* (Willd.)DC	Shikaakaai	
7.	*Sapindus mukorassi* Gaetrn	Boonthikottai	
8.	*Rosa species* L	Rose	

Table 2: Herbal shampoo ingredients and their usage (Jacob *et al.*, 2015)

Sr.No.	Name of the herbs	Parts used	Quantity	Uses
1.	*Hibiscus rosa – sinensis* L	Leaves and flower	Each 100g	Hair growth
2.	*Trigonella foenum – graecum* L	Seeds	50 g	Hair conditioner
3.	*Lawsonia inermis* L	Leaves	100g	Reduce hair fall and improve hair colour
4.	*Murraya koenigii* L	Leaves	100g	Remove dead hair follicles
5.	*Aloe barbedensis* L gel	Gel	50g	Dry hair and skin
6.	*Acacia concinna* (Willd.)DC	Fruit (Pod) and seeds	25g	Scalp health and reduce itching, lice
7.	*Sapindus mukorassi* Gaetrn	Fruit (Pod) and seeds	25g	Reduce dirt
8.	*Rosa species* L	Petals (Rose water)	50 ml	Hair growth and protect skin, perfumery

6. Skin irritation test:

Herbal shampoo was examined by applied in the hair and wait for 5 minutes and changes noticed in skin colour or itching in scalp. All ingredients are added naturally and never damage skin texture (Saraf, 2011).

7. Eye irritation test:

Herbal shampoo was test by while applied in the hair, it is irritated or eyes are reddish in colour. Most of the chemical based shampoo causes eye irritation. Phytochemicals protect our eye from dangerous condition (Valavai *et al.*, 2017).

8. Conditioning attributes:

The conditioning effect of the shampoo react on the hair was evaluated after washing the hair and conditioning properties include all desirable benefits increased to hair.

Result and Discussion:

The herbal products are used as medicine and as well as cosmetic purpose. Different products are used to protect skin, hair colour, texture and conditioner. These ingredients are safer for hair care.

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Table 3: Physical appearance test (Valavai *et al.*, 2017)

Sr. No.	Organoleptic appearance	Result
1.	Colour	Brown
2.	Odour	Aggreable
3.	Taste	Distinctive
4.	Texture	Shiny

Table 4: Evaluation of herbal shampoo (Vijetha *et al.*, 2013)

Sr. No.	Physicochemical properties	Result
1.	P^H value	5.8
2.	Wetting time	3 min 12 sec
3.	Foaming stability	Good foam
4.	Dirt dispersion	moderate
5.	Skin irritation	No irritation or rashes on skin
6.	Eye irritation	No irritation or reddishness
7.	Conditioning attributes	Soft, shiny

Herbal shampoo:

Herbal shampoo enriches the hair quality and texture. The above tests were examined under various analytical aspects. Traditional products are easily available in home and having high medicinal value, so can be used as cosmetic products (Valavi *et al.*, 2017).

Conclusion:

The herbal shampoo are playing vital role in human life now a days. People turning back to nature, so herbal products usage increased in World wide. Herbal shampoos are not only

removing dirt, it is safer in use, improving hair growth, condition, protecting scalp and eco - friendly. In this present study all ingredients are herbs with few medicinal values; Polyherbal mixing is a giving great impact on hair. The above evaluation method like Physical appearance and Physicochemical Evaluation are shown the quality of shampoo. Herbal products never cause any side effects with proper ratio leads to be a healthier body condition. In future there are chances to use more herbs and their by products in the commercial production by hair care industries.

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NUTRITIONAL COMPOSITION OF LATEX FROM REDINA LETTUCE PLANT (*LACTUCA SATIVA L*-RED VARIETY)

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Abstract:

Plant latex has been found to play an important role in plants defence against insect herbivores. In the last two decades, the interest received by plant latex has been on the rise because of its anthelmintic activity. In the present investigation the latex was collected from young stem and leaves of nutrition rich red leaf lettuce plant (Red variety) variety of *Lactuca sativa L.* Characterization of the latex for the biochemical compounds such as protein content, iron (II) and iron (III) by spectrophotometric method, potassium by flame photometry, calcium by complexometric titration vitamin C by redox titration was carried out. The protein content in the latex was found to be 18 mg/Kg. Total iron content in the latex from 1 Kg lettuce contains 0.072 mg. Electrolyte like potassium was detected by flame photometry revealed 0.08 mg/Kg. Calcium and vitamin – C content was found to be 0.6 mg and 3.1 mg/Kg. Hence the above investigation was carried in order to explore the importance of nutritional composition of *Lactuca sativa L* and in order to depict that there is nutrients present in addition to alkaloids, starches, sugars, oils, tannins, resins and gums are found.

Keywords: *Lactuca Sativa L*, Nutrients, Latex**,** Protein, Spectrophotometry, Antioxidants.

Introduction:

Lettuce is one of the oldest and most popular leafy vegetable originated from the Mediterranean region (Balsam *et al.* 2013). Lettuce (*Lactuca sativa*) is one of the most widely produced and consumed leafy greens as it is easy to prepare, easily available, and has numerous health promoting properties (Kim *et al.* 2016). The name lettuce and botanical name *Lactuca* came from the Latin word lac means milk and refers to the milky sap present inside lettuce leaves and stems. Although lettuce has high water content and is low in calories, it enriches the human diet with dietary fiber and important mineral nutrients, such as vitamin A, vitamin K and

potassium, with higher concentrations of vitamin A found in darker green lettuces. It also provides some dietary fiber, carbohydrates and protein (Kim *et al.,* 2016). The consumption of nutrient-rich vegetable products can significantly counter human health problems like obesity and chronic disorders, including diabetes or cardiovascular disease (Bazzano *et al.*, 2002, Hung *et al.*, 2004). With the exception of the iceberg type, lettuce also provides some vitamin C, calcium, iron and copper, with vitamins and minerals largely found in the leaf (Cristofano *et al.*, 2021). Lettuce naturally absorbs and concentrates lithium. In addition to its usual purpose as an edible leafy vegetable, lettuce consumers benefit from a variety of health improvements, starting from the general lowered risk of diseases due to the consumption of vegetables (Vaštakait-Kairien *et al.*, 2021) and the elevated intake of phytochemicals such as vitamins, polyunsaturated fatty acids (PUFA) and antioxidants pertained to this leafy vegetable (Nicole *et al.,* 2004). Lettuce extracts are sometimes used in skin creams and lotions for treating sunburn and rough skin. It was once thought to be useful in relieving liver issues.

Red leaf lettuce:

Figure 1: Red leaf lettuce

Red leaf lettuce produce latex refers generically to a stable dispersion of polymer micro particles in an aqueous medium. Many plants that coagulate on exposure to air and in most plant, latex is white, but some have yellow, orange or scarlet latex. It is a complex emulsion in which proteins; alkaloids, starches, sugars, oil, tannins, and gums are found (Wesolowsk et. al. 2006). The lettuce contain the enzymes called proteases namely lettucine has been isolated and characterized from *Lactuca sativa L* leaves showed caseinolytic activity and milk clotting activity (Angela *et al.* 2002). They also exhibit anthelmintic activity against insectivores. Important latex producing plants belongs to the following family, Namely – Apocynaceae, Asclepiadaceae, Cannabinaceae, Caricaceae, Compositae, cnvolulaceae, Sapotaceae etc. These are five main classes of lettuce and over a hundred of various types, including typical ‘Iceberg’,

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Greek Lakes', 'Boston', 'Butter crunch' or 'New York', 'Red rosy', small 'Mini green' or 'Tom Thumb' etc. Some lettuce plants have a rosette of broad soft leaves or long sword-Shaped leaves (Mampholo *et al.,* 2016). Some have cabbage heads with crispy leaves, some have loose head with puffy inner leaves and some have edible stems (Thomas 2021; Byrdwell *et al.,* 2021; Miaone *et al.,* 2020). There are 4 main types of Lettuce; Crisp head lettuce 2. Romaine or Cos lettuce 3. Butter head 4. Celtuce lettuce 5. Red leaf lettuce. Red leaf lettuce is a variety of green leaf lettuce and one of 100 different lettuce varieties grown worldwide. Unlike iceberg lettuce, red leaf lettuce is a loose-headed variety with long leaves (Kim *et al.,* 2016). The name can be a little deceiving since red leaf lettuces are not completely red. Instead, the top of the leaves exhibit some red or purple coloring that may extend a few inches into the leaf. Red leaf lettuce has a mild, watery flavor with soft green leaves that are tinged with a reddish color. Leaf lettuces have more flavor than head lettuces, and red leaf adds a vibrant splash of bronzy red to a salad.

Nutritional and Medicinal uses of red leaf lettuce:

Red leaf lettuce packs a high nutritional value, is an excellent source of beta carotene, which may be indicated in reducing risk for developing cataracts (Gil, 2015). Kim and his co-workers have reported nutritional value of lettuce greatly varies with lettuce types depending on lettuce type, and nutrient composition can be equivalent to other "nutritious" vegetables (Kim, Moon, *et al.,* 2016). A red leaf lettuce belongs to family Asteraceae, to the genus and species *L. sativa*. It has the advantage too, of adding some very appealing color to a salad. Sandwiches can be made that much more special with a few delicately layered red. Red is high in vitamin A and other antioxidants (Baslam *et al.,* 2013; Lanza Volpe *et al.,* 2021). Vitamin K has a potential role in the bone metabolism where it thought to increase bone mass by promoting osteotrophic activity in the bone cells. It also has established role in Alzheimer's disease patients by limiting neuronal damage in the brain. Fresh leaves contains good amount of folates and vitamin C. Folates require for DNA synthesis. Vitamin C is a powerful natural antioxidant (Li *et al.,* 2021, Xu *et al.,* 2017, Zhang *et al.,* 2016). Zea-xanthin is an important dietary carotenoid in lettuce. It is selectively adsorbed into the retinal macula lutea, where it thought to provide antioxidant and filter UV rays falling on the retina. Potassium is an important component of cell and body fluid that helps controlling heart rate and blood pressure (Byrdwell *et al.,* 2020). Beneficial health properties of lettuce have mainly been attributed to carotenoids and other phytochemicals such as phenolic compounds (López *et al.,* 2014). Manganese is used by as a co-factor for antioxidant enzymes, superoxide dismutase. Copper is required in the production of red blood cells. Iron is

essential for red blood cell formation. It is rich in B-complex group of vitamin like thiamine, vit-B_6 and riboflavin (Samtiya and Aluko *et al.,* 2021). Regular inclusion of lettuce in salads is known to prevent osteoporosis, iron-deficiency anemia and believed to protect from cardiovascular disease, Alzheimer's disease and cancer. The objective of this work was to look for some of its nutrients composition in latex of this plant having health benefiting nutrients. In the present investigation, nutritional value in latex was evaluated in the red lettuce plant (contains protease enzyme having anthelmintic activity).

Materials and Method:

Chemicals:

General chemicals and solvents used were of analytical grade obtained from RANKEM. They are potassium chloride, ammonium molybdate, copper sulphate, FAS, thiocyanate, 1, 10-phenanthrolein, acetic acid, sodium acetate, Ce (IV) sulphate, Zinc Sulphate, EDTA, ammonium chloride etc.

Latex collection from plant:

One kilogram of fresh Red leaf lettuce was obtained from the local market. The roots and young stems of the plant were washed thoroughly. Immediately latex collected from the young stems in 0.01 M sodium acetate buffer of pH 4.0. The collected latex was kept in freezer. Before using for analysis it was thawed to room temperature, the latex was centrifuged and the supernatant was used for further analysis.

A) Determination of Protein content of latex:

Protein content in the partially purified enzyme was carried out by Lowry's method. For some time it was the method of choice for accurate protein determination for cell fractions, chromatography fractions, enzyme preparations and so on. The method developed by Lowry *et. al.* (1951) is sensitive enough to give a moderately constant value and hence largely followed. Protein content of enzyme extracts is usually determined by this method. Under alkaline conditions, the divalent copper ion forms a complex with peptide bonds in which it is reduced to a monovalent ion. Monovalent copper ion and the radical groups of tyrosine, tryptophan, and cysteine react with Folins – Ciocalteau reagent to produce an unstable product that becomes reduced to molybdenum/tungsten blue. Into a series of 25 mL volumetric flask 0.5, 0.1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 mL of BSA was added. The solution was made up to 1mL by the addition of 0.1 N NaOH. Meanwhile, unknown was prepared by adding 0.1mL and 0.2 mL of latex supernatant and made up to 1mL with NaOH. To each of this 5 mL of alkaline copper sulphate solution was added, mixed well and allowed to stand for about 10 min. Then 0.5 mL of

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Folin's reagent was added and allowed for 30 min. The absorbance of this solution was measured at 340 nm using Systronics Visiscan 167 Spectrophotometer. Then a calibration curve was drawn as a function of absorbance v/s concentration of BSA. From this calibration curve the amount of protein in the sample was determined.

B) Determination of Iron (II) by Spectrophotometric method:

Iron (II) forms a stable, highly colored complex with 1, 10 – phenanthrolein:

$$Fe^{2+} + 3\,(ph)H^{+} \rightarrow Fe\,(ph)_3^{2+} + 3H^{+}$$

Iron (III) does not form the complex. For this analysis to be successful, all of the iron must be reduced from Fe (III) to Fe (II). In this experiment (Mendham *et al.* 2006), the reduction is carried out with hydroxylamine hydrochloride, while the solution is buffered with sodium acetate. Calibration curve was prepared by pipetting, 0.4, 0.8, 1.6, 2.0, 2.4 mL of 0.5×10^{-3}M FAS solution into a series of 10.0 mL standard flask. Unknown was prepared by adding 0.2 and 0.4 mL of latex supernatant. To each of the flask 4.0 mL of 1, 10-phenanthroline and 3.0 mL of buffer was added, kept aside for 10 min for colour development. Once the colour developed, solutions were diluted up to the mark using distilled water, shaken well for uniform concentration. The percentage transmittances of these solutions at 510 nm were measured using Systronics visiscan 167 spectrophotometer. A graph of concentration of iron (II) along X-axis and absorbance along Y-axis was plotted, from this concentration of unknown was determined.

C) Spectrophotometric Determination of Iron (III) by Thiocyanate Method:

Ferric ion reacts with thiocyanate to give a series of intensely red coloured compound which remains in true solution. Ferrous ion does not react, depending upon the thiocyanate concentration series of complex are formed as $(Fe(SCN)_4)^{-}$ where n = 1,2,3 ….6 at low thiocyanate concentration, the predominant colored species is $(Fe\,(SCN))^{2+}$**.**

$$Fe^{3+} + SCN^{-} \rightarrow (Fe\,(SCN))^{2+}$$

At 0.1 M thiocyanate concentration it is largely the complex formed is **(**$Fe(SCN)_2$**)+,** at very high thiocyanate concentration, the complex formed is **(**$Fe(SCN)_6$**)**$^{3-}$, in the spectrophotometric determination a large excess of thiocyanate should be used, since this increases the intensity and also stability of the color. Strong acid should be present to suppress the hydrolysis.

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^{+}.$$

H_2SO_4 is not recommended because sulphate ion have a certain tendency to form a complex with Ferric ions, Silver, Copper, Nickel, Titanium, Uranium, Molybdenum, Mercury, Zinc, Cadmium, Bismuth interfere, mercurous and stannous salt if present must be converted into mercuric and stannic salt otherwise the color is destroyed. Phosphate, arsenate, fluorides, oxalates interfere,

since they forms fairly stable complex with ferric ion the influence of phosphate and arsenates can be reduced by use of concentrated acids. In to a series of 25 mL volumetric flask 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 up to 5ml of ammonium ferric (lll) sulphate solution were added using micro burette. In another flask samples 0.2 and 0.4 mL were taken, 2.5 mL of 20 % KSCN solution and 3 mL of HCl were added into each flask and allowed to stand for few minutes, then solutions were made up to the mark using distilled water and shaken well, the absorbance were read at 480. The absorbance of the sample was recorded and concentration was determined with a calibration curve.

D) Determination of Potassium by Flame Photometry:

Flame photometer or atomic emission spectroscopy is used for the analysis of element that exhibits an easily excited flame spectrum of sufficiently intensity for the detection by a photo cell. The region of the spectrum appropriate to the element being determined is isolated by means of optical filter. The method of extraction of potassium in the flame as follows. The water evaporates in the KCl solution, leading behind minute particles of the salt or the mixture of salt, at higher temperature, KCl decompose and vaporised to give constituent atom. The vapour of K atom is then exited by the thermal energy of the flame finally the resulting emission. Pipette out 0, 0.8, 1.6, 2.4, 3.2 mL of 1000 ppm potassium solution into six different 10 mL volumetric flask These solutions were made up to the mark, mixed well. Readings was noted down in flame photometer. Unknown solution prepared by taking 0.2 mL of latex supernatant and made up to 10 mL. A graph of concentration in ppm of potassium against meter reading was plotted. From the graph the concentration in the latex sample was determined.

E) Determination of Calcium in Latex:

Ethylene diamine tetra acetic acid is a hexadentate ligand. It has six potential sites for binding with metal ion. In strong basic solution all the 4-COOH groups are deprotonated and forms 1:1 complex with a variety of multivalent metal ions like Mg^{2+}, Ca^{+2}, Ba^{2+} etc. The indicator used is EBT. In the range pH 7-11 the indicator is blue in colour. In the pH range bellow 5.5 the indicator is red in colour. To the metal indicator complex which is wine red in colour if EDTA added, will displace the indicator from the metal indicator complex and forms metal EDTA complex just offer the endpoint. i.e., the metal indicator is pale blue colour. Patton Reeder's indicator makes it possible to determine the Ca alone in the presence of Mg content in the H_2O sample.

i) **Standardisation of EDTA solution**: Pipette out 10 mL of 0.01M standard $ZnSO_4$ solution into clean conical flask. Add 1 mL of buffer solution (pH=10), 15 mL

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distilled water and add a drop of EBT indicator and titrated against EDTA taken in the burette, till blue color appear. The titration was repeated for concordant value.

ii **Determination of calcium concentration in latex**: About 0.2 mL of latex extract was made upto 10 mL with distilled water. About 3 mL of 8 M KOH was added to maintain pH at 11 followed by a pinch of Patton Reeder indicator. Solution turns wine red colour. The solution was titrated with the standard EDTA solution until the colour changes to blue. The amount of Ca present in the extract sample calculated using the relation - 1 mL of 1 M EDTA = 40 mg of Ca.

F) Determination of Vitamin – C:

Vitamin-C (L-ascorbic acid) get oxidised to its dehydro form by air especially at alkaline pH. However it is stable in an acidic solution. Its estimation in the extract is carried out by titrating it against Ce (IV) sulphate solution using ferroin as an indicator. Being a strong oxidising agent Ce (IV) oxidises Vitamin-C in an acidic medium to its de hydro form. The redox reaction occurring during titration when 2, 6 - dichloro phenol indophenols and also Ce (IV) sulphate is used as a titrant.

i. **Standardisation of Ce (IV) solution:** 10 mL of 0.01 M FAS was pipette into clean 100 mL conical flask, 2 mL of 2M H_2SO_4 added and titrated against Ce (IV) solution taken in the burette using ferroin as an indicator, till the colour changes from orange red to pale green. Titration was repeated to get concordant volume. Titer values were recorded. From the titer value concentration of Ce (IV) sulphate solution was calculated.

ii. **Estimation of Vitamin-C in latex using Ce (IV) solution:** About 0.2 mL of latex supernatant was taken into a 100 mL conical flask and diluted to 5 mL with distilled water. To this 2 mL of 2M H_2SO_4 and 2 drops of ferroin indicator was added, titrated against standardised Ce (IV) solution taken in the burette till the colour changes from orange red to pale green. Titration was repeated to get concordant value.

Results and Discussion:

In the present study, the latex was collected from 1 Kg of red variety lettuce and suspended in 0.01 M sodium acetate buffer of pH 4. Latex collected from young stem and leaves were analysed for nutrients such as protein, iron (II), iron (III), vitamin-C, calcium and potassium. The protein content in the latex was determined by Lowry's method by

spectrophotometer as shown in the table 1. The protein content was found to be 3.0 mg/mL. The latex collected from 1 Kg lettuce which was about 6 mL contains 18 mg of protein; where as 100 g green leaf (Crispy head) contains 1.36 g protein (United States Department of Agriculture - USDA nutrient database, 2015).

The latex contain about 14.4 µg of iron (II) determined by 1, 10 phenanthroline as shown in the fig. 2, and about 58 µg of iron (III) was determined by thiocyanate method as shown in fig. 3, Hence the total iron content in the latex from 1 Kg lettuce contains 0.072 mg, while green leaf crispy head variety contains about 0.86 mg of iron in 100 g of lettuce leaf.

Table 1: Protein content of raw and cooked rhubarb sample

Sr. No.	Protein solution in mL	Conc. µg	Alkaline CuSO4 solution mL	Foling's reagent	Incubation time (mins)	Absorbance at 660 nm
1	0.05	50	5	0.5	5	0.158
2	0.10	100	5	0.5	5	0.294
3	0.15	150	5	0.5	5	0.482
4	0.20	200	5	0.5	5	0.674
5	0.25	250	5	0.5	5	0.842
6	0.30	300	5	0.5	5	0.993
7	Sample 1	-	5	0.5	5	0.174
8	Sample 2	-	5	0.5	5	0.163

The Vitamin-C content determined by redox titration, latex collected from 1 Kg lettuce exhibited 3.1 mg, where as 100 g crisp head green leaf contains 9.2 mg (United States Department of Agriculture -USDA nutrient database, 2015). The Ca^{2+} content of latex from 1 Kg lettuce was about 0.6 mg where as 36 mg of calcium has been found in green leaf Crispy head variety lettuce.

Electrolyte like potassium was detected by flame photometry revealed 0.08 mg of K^{+} as shown fig. 4, and high content of potassium about 194 mg has been reported in 100 g green leaf lettuce (United States Department of Agriculture -USDA nutrient database, 2015). From the above analysis, it is indicated that lettuce not only contains phytochemicals like alkaloids, antioxidants but also nutrients may be in lesser amounts.

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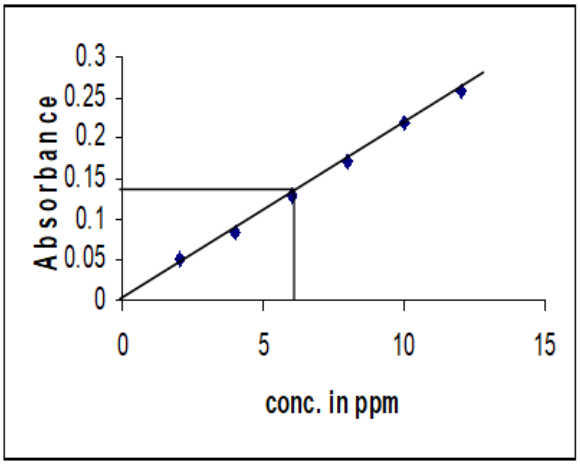

Figure 2: Calibration curve for determination of Fe (II) by spectrophotometric method

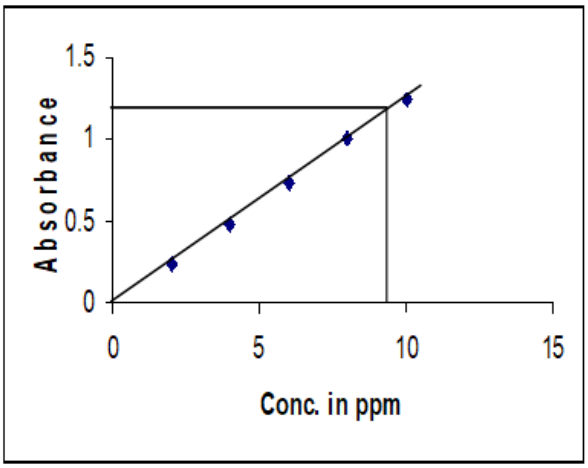

Figure 3: Calibration curve for determination of Fe (III) by spectrophotometric method

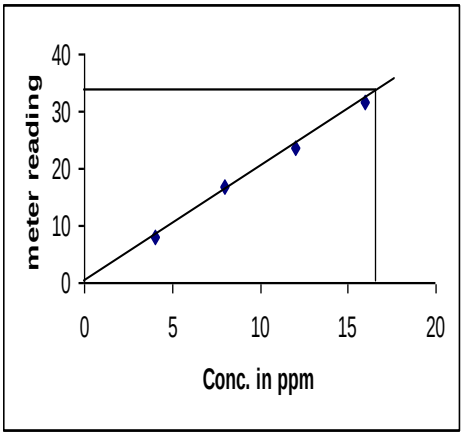

Figure 4: Calibration curve for the determination of Potassium by Flame photometric method

Summary and Conclusion:

In the present investigation the latex was collected from 1 Kg of red variety lettuce (*Lactuca sativa)*. Latex collected from young stem and leaves were analysed for nutrients such as protein, iron contents, vitamin-C, calcium, and potassium. Its protein content in the latex was determined by Lowry's method by spectrophotometer and was found to be 3.0 mg/mL. The latex content of iron (II) was determined by 1, 10-phenanthrlien and iron (III) determined by thiocyanate method. Iron contents was found to be found to be 0.072 mg. Electrolyte potassium was detected by flame photometry and was found to found to be 0.08 mg respectively. Calcium

content determined by complexometric titration was found to be 0.6 mg and vitamin-C content was determined by redox titration was 3.1 mg. The above results indicated that the latex also contains nutrients may be in lesser amounts in addition to alkaloids, starches, sugars, oils, tannins, resins, gums and protease enzyme.

List of abbreviations:

FAS – Ferrous Ammonium Sulphate
EDTA – Ethylene Diamine Tetra Acetate
BSA – Bovine Serum Albumin
SCN – Thiocyanate
EBT – Eriochrome Black T
Ph – Phenanthrolein
$[Fe(SCN)]^{2+}$ – Ferric thiocyanate
ppm – parts per million
Kg – Kilogram
mg – milligram
mL – milliliter

Chemicals Molecular Formulae:

KOH – Potassium hydroxide
H_2O – Water
HCl – Hydrochloric acid
H_2SO_4 – Sulphuric acid
Ce (IV) – Cerium IV sulphate
$ZnSO_4.7H_2O$ – Zincsulphate heptahydrate
COOH – Carboxylic acid
KSCN – Potassium thiocyanate

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MICROBIAL BIOFERTILIZERS FOR AGRICULTURAL SUSTAINABILITY

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Abstract:

The uncontrolled and immense exploitation of chemical fertilizers has causedmany environmental problems such asozone layer depletion, greenhouse effect, infertility of land, biotoxification, bioaccumulation, acidification of water etc. These problems can be reduced by replacing chemical fertilizers withmicrobial based fertilizers and pesticides.Microbial based fertilizers are natural, beneficial, and ecologically and user-friendly. The biofertilizers provide nutrients to the plants, control soil borne diseases, and maintain soil structure hence act as a good soil conditioner. Microbial based fertilizers play a considerable role in sustainable agriculture. A good crop yield can be maintained by using the biofertilizers based on *Azotobacter* sp., *Rhizobium* sp., Arbuscular mycorrhizal fungi (AMF) and phosphate solubilizing bacteria (PSB). A group of growth promoting nitrogen-fixing bacteria is universally exploited as biofertilizer. Moreover, a sustained agricultural productivity has been achieved by the use of *Rhizobium*sp. in legume crops. The response of two efficient bacteria viz. *Azotobacter* sp. and *Azospirillum* sp. has been universally experienced for the improvement in crop yield. Arbuscular mycorrhizal fungi (AMF) can form symbiotic associations with most of the terrestrial plants. These fungi are mainly responsible for phosphorus uptake and beneficial when inoculated at the seedling stage of plant growth. On the other hand, a group of phosphate solubilizing bacteria is immensely important since these bacteria convert insoluble forms of phosphorus into soluble ones hence promote uptake of phosphorus by plants.Thus microbial based fertilizers help to gain higher yield of crops, vegetables, and trees and reduces environmental problems. In this chapter, we have reviewed the types of biofertilizer, production strategy and their advantages.

Keywords: Biofertilizer, Azotobacter, Rhizobium, mycorrhizal fungi, agricultural sustainability

Introduction:

The increasing use of chemical fertilizers has created environmental problems such as deterioration of soil quality, surface water, and groundwater. This has also caused air pollution, reduced biodiversity, and suppressed ecosystem function. Use of biofertilizer provides an inexpensive and attractive alternative in farming. The most limiting nutrients for plant growth are nitrogen and phosphorus. Soil contains nitrogen and phosphorus however they not readily available for plant use (Rai, 2006). Phosphorus is one of the major essential macronutrients and has an important role in several metabolic processes like photosynthesis, respiration, root elongation, seed development. Phosphorus deficiency results in the leaves browning, weak stem and slow development. The concentration of soluble phosphate in soil is very low which make it limiting factor in plant nutrition (Rai, 2006; Hingole and Pathak, 2013, 2015, 2016a, 2016b, Sabalpara and Mahatma, 2019).

Symbiotic nitrogen fixing bacteria as biofertilizers

Rhizobia and Frankia are symbiotic nitrogen fixing microorganisms.Rhizobia belong to the family rhizobiaceae and include the genera viz. *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium*, and *Allorhizobium*. These bacteria infect to legumes. The nitrogen fixing capability of rhizobia has reported up to 450 Kg $N \cdot ha^{-1}$. Use of biofertilizer on legumes is an old practice carried out for more than a century in agricultural systems of United States and the United Kingdom nations (Rai, 2006). Use of rhizobia as bioinoculant is important when resident soil rhizobial populations are either absent or known to be very low. The rhizobialinoculum can be produced and applied in number of ways. *Frankia* is the genus of symbiotic nitrogen-fixing actinomycetes. Many species from the genus Frankia are able to infect a group of eight families of mainly woody plants. However, only limited information is available for inoculation practices and the use of *Frankia* sp (Bhattacharjee *et al.,* 2008; Gupta *et al.,* 2012).

Non-symbiotic nitrogen fixing bacteria as biofertilizers

Many non symbiotic nitrogen fixing bacteria have been exploited as biofertilizer; however Azotobacter sp. is used as apotential bio-fertilizer for soil and plant health management**.** In addition, application of this bacterium has also become helpful in the reclamation of soil by transforming virgin land to fertile one (Sumbul *et al.,* 2020). *Azotobacter* is a group of Gram negative, free-living, nitrogen fixing aerobic bacteria inhabiting in garden soil. They form thick-walled cysts i.e. dormant cells resistant to unfavorable environmental conditions, making them, in situ useful candidate as biofertilizer. *Azotobacter* sp. is able to convert atmospheric nitrogen to ammonia, which in turn is taken up and utilized by the plants. Owing to its ability to improve

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plant health not only through nitrogen fixation but also by growth hormone production, phosphate solubilization, plant disease management and reclamation of better soil health, *Azotobacter* sp. is one of the best options to be used as biofertilizer for sustainable agriculture (Rai, 2006; Jnawali *et al.,* 2015).

Cyanobacteria as biofertilizers

An aquatic cyanobacterium, *Trichodesmium,* contributes approximately 36% of global nitrogen fixation. In many regions of Asia, the *Nostoc*sp.and *Anabaena* sp. of cyanobacteria still has shown remarkable results for rice cultivation. Moreover, cyanobacteriahave shown beneficial effects in field-cultivation of rice, paddy, wheat, soybean, tomato, radish, cotton, maize, sugarcane etc. (Vaishampayan *et al.,* 2001).Cyanobacterial species are differentiated on the basis of their specialized structure forming ability i.e. heterocyst. The heterocyst formers are *Aulosira*, *Anabaena*, and *Nostoc* sp. Some species are non-heterocystous, for example, *Aphanothece*, *Gloeocapsa*, and *Gloeothece* sp. Some cyanobacterial species are filamentous, for example, *Oscillatoria*sp. and *Plectonema* sp. The heterocystous and filamentous cyanobacteria are considered as potential biofertilizers. Heterocystous and non-heterocystouscyanobacteria are categorized as symbiotic and free-living (non-symbiotic), respectively. *Anabaena*, *Nostoc*, *Aulosira*, *Tolypothrix*, *Cylindrospermum*, and *Stigonema* sp. are chlorophyll-containing, photosynthetic and free-living (non-symbiotic) prokaryotic cyanobacteria, also called as blue green algae. In agriculture, cyanobacterial biomass is directly used as bioinoculants to increase the crop productivity (Rai, 2006; Saadatnia and Riahi, 2009).

Phosphate solubilizingmicroorganisms as biofertilizers

After nitrogen, phosphorus is the second significant element required in plant nutrition. Phosphorus is commonly found in the form of polyprotic phosphoric acid (H_3PO_4); however, phosphorus intake is naturally in the form of H_2PO^{-4} (Menezes-Blackburn *et al.,* 2016). Phosphorus is present in various forms in soil i.e. inorganic phosphorus, organic phosphorus, adsorbed phosphorus, and mineral phosphorus; of these only the former first form is available to plants. Complex organic phosphate compounds, for example, nucleic acids, phospholipids, etc. are abundantly found in soil, hence they have to be transformed by microorganisms (Yadav *et al.,* 2017). The phosphorus mineralization process in the soil involves the production of microbial enzymes, such as phosphatases and phytases. Phosphate solubilizing and mineralizing features are found in some species of *Acetobacter* sp., *Aspergillus flavus*, *Bacillus* sp., *Penicillium* sp., *Aspergillus niger*, *Burkholderia cepacia*, *Burkholderia* sp., *Serratia* sp., *Ralstonia* sp., *Pantoea* sp., *Citrobacter* sp., *Enterobacter* sp., *Escherichia freundii*, *Penicillium*

bilaii, Penicillium regulosum, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas striata, Rhizobium leguminosarum, Serratia marcescens, Sinorhizobium meliloti and *Stenotrophomonas maltophilia*. These phosphate-solubilizing microbial strains are commercially offered in formulated products that are cast off as biofertilizers. Alginate gel is used in fungal and bacterial bioformulations. Gram positive spore forming phosphate solubilizing bacteria are mostly preferred in bioformulations, since spores of such bacteria are heat resistant and could survive in harsh conditions. Bioformulation of several Gram negative phosphate solubilizing bacteria is problematic since they do not produce spores. In spite of that, a few industries are commercializing bacterial stains as biofertilizers, for example, bacterial bioformulation of *Pseudomonas aureofaciens* has been commercialized by Ecosoil. Some commercially available phosphate-solubilizing bioformulation inoculants are sold in India under trade name, for example, Gmax Phosphomax, Kisan PSB, Astha PSB, Gmax Tricon, SKS TV, Gmax FYTON, Astha PF, SKSPF, Gmax Sugarmax, UPAJ-K, Eco-Potash, UPAJ- Z, BioZinc, zinc-cure, AgriVAM, bio e rich, Novozymes, Ecosoil, Anubhav liquid bioformulation, SKS VL etc (Rai, 2006; Khajeeyan*et al.,* 2019; Yadav *et al.,* 2020).

Mycorrhizal Fungi as biofertilizers

The keyword mycorrhiza reflects a symbiotic association between plant roots and fungi. In the mycorrhiza association, the fungi colonize the plant root either intracellularly or extracellularly, depending on the type of plant and fungus involved in the association. Simply, the interaction occurs between a host plant and fungus in the sense that the fungus is supplied with carbohydrates by host plants for its metabolic activities, and, in exchange, the host plant is supplied with nutrients and water needed for its growth by the fungus. Thus, the association between the fungus and the host plant is a mutually beneficial hence called as symbiotic association. Moreover, mycorrhizal fungi can facilitate the detoxification of organic and inorganic soil pollutants. There are two types of mycorrhizal fungi namely, endomycorrhiza and ectomycorrhiza. Endomycorrhizalhyphae penetrate cortical cells of plant root and forms intracellular arbuscule. This type of arbuscule formation has been reported in 86% of plant species. Ectomycorrhizalhyphae do not penetrate plant root cells. Many researchers have reported contribution of arbuscular mycorrhizal fungi in plant growth promotion. *Glomus versiforme* and *Glomus mosseae* infect to tomato plant roots, forms arbascules and promote growth of this plant even in water stress conditions. *Glomus etunicatum* infects to maize plant root cells and as consequences, improvement in chlorophyll content and nutrient uptake in maizeplant takes place and soil quality also increases in a greater extent (Krishnamoorthy *et al.,* 2016). *Acaulospora lacunosa* infects to strawberry plant root cells and then helps to enhance

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nutrient uptake by the plant. The effects produced by a mycorrhizal fungus *Rhizophagus irregularis* in wheat plants are improvement to stress tolerance, enhancement in plant growth, and increment in seed yield. In this way, the mycorrhizal fungi *R. irregularis, G. geosporus, Rhizophagus irregularis, Glomus deserticola, Mortierella* sp. And *Glomus versiforme* have been proven beneficial for maize, strawberry, tomato, snapdragon, seashore mallow and *Mentha arvensis* L. plants respectively, by producing effects such as enhancement in growth, improvement in nutrient uptake in salt stress conditions, increment in dry weight of shoot and root in poor salt condition and protection by some pathogens (Rai, 2006; Mora-Romero *et al.,* 2015; Boyer *et al.,* 2015; Tognon *et al.,* 2016; Tohidi *et al.,* 2018; Xu *et al.,* 2019; Chiomento *et al.,* 2019; El Maaloum *et al.,* 2020).

Carrier materials, formulation and field application of biofertilizer

Carrier materials are classified into five categories namely, natural material, inert material, synthetic polymer, natural polymer, organic material and agro-industry by-product (Shravani, 2019). Peat, lignite, coal, clay, and organic soil are the types of natural materials. Talc, vermiculite, perlite kaolin, bentonite, silicate, rock phosphate, calcium sulfate, and zeolite are used as inert materials. Polyacrylamide, polystyrene, and polyurethane are synthetic polymers. Xanthan gum, carrageenan, agar agar, and agarose are some natural polymers. Charcoal, biochar, composts, farm yard manure, sawdust, maize straw, vermicompost, cow dung, corn cob, and wheat husk are the types of organic materials. Sludge ash and jagerry are the types of agro-industry by-products. Selection and preparation of a best carrier is a critical step in bioformulation of inoculants (Herrmann and Lesueur, 2013; Roychowdhury *et al.,* 2015; Hassan and Bano, 2016; Thirumal *et al.,* 2017). Inoculum can be prepared in powder, liquid, and granular forms. Generally, granular formulations are convenient. The use of pre-sterilized carrier such as coco peat, perlite, mineral soil, charcoal etc. is another important feature of biofertilizer inoculants. Low density of infective propagules and inadequate storage conditions are the reasons of getting low yield. Hence it is important to control the quality of the prepared biofertilizers. Green house trials, field validation, quality control, maintenance of storage conditions etc. are some important aspects that should be assessed critically through-out the whole process. Experts can recommend the use of biofertilizer to farmers based on soil quality analysis report and the crop selected for cultivation. The field application of biofertilizers under a trained supervisor reduces mistakes and thus helps to yield better productivity of selected crops (Rai, 2006; Rodrigues andRodrigues, 2017; Paliya *et al.,* 2019; Bharathi *et al.,* 2004;

Saravanakumar *et al.*, 2010; Fasusi *et al.,* 2021). General production strategy and field application of a biofertilizer is given in Figure 1.

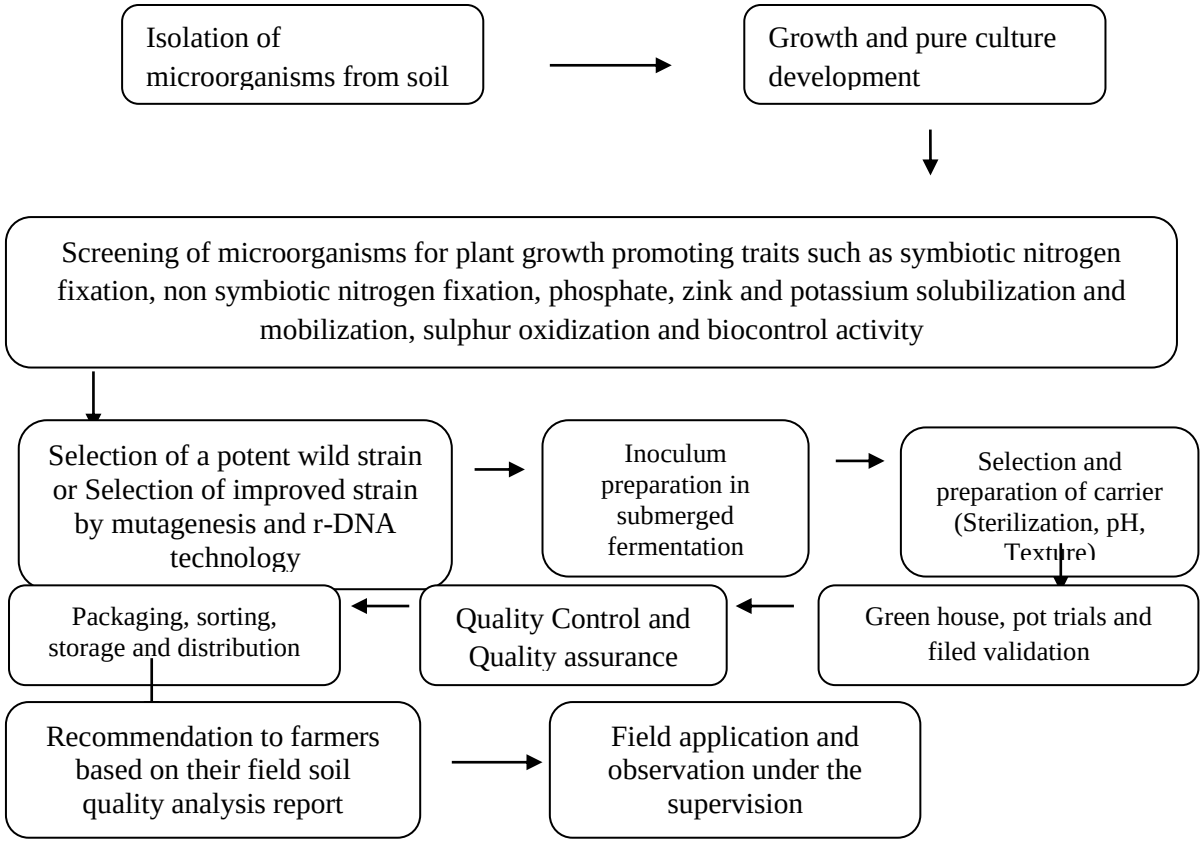

Figure 1: Simplified flowchart of general production strategy and field application of a biofertilizer

Conclusion and future prospective:

Biofertilizers are the products composed of viable strains of microorganisms used to promote and enhance plant growth without causing harm to human health and the environment. Different biofertilizers show varying types of effects for different soils; hence, the utilization of biofertilizer depends upon the type of land and crops selected for cultivation.Microbial nitrogen fixation, phosphate solubilization and mobilization, and sulphur oxidation are important biological processes for harnessing health of soil and assuring agronomic, economic, and environmental benefits. A lot of hard work in the extreme extent is still to be carried out as numerous potential nitrogen fixing, phosphate solubilizing and sulphur oxidizing microorganisms, have been identified by researchers and they haven't up till now commercialized resourcefully.

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FILAMENTOUS CYANOBACTERIA FROM THE SOILS OF KARSA – POHREGAON BARRAGE LATUR, MAHARASHTRA

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Abstract:

The Karsa-Pohregaon barrage is constructed on Manjara River at Pohregaon in 2008. The present investigation was carried outduring April 2017 to March 2018 to study thecynobacterial diversity from the soilsof barrage in Renapur tehsil of Latur districtin the Marathwada region of Maharashtra.A total of 82 taxa under 47 genera belonged to blue greens were encountered. The present paper deals with the systematic enumeration of only filamentous cyanobacterial forms like *Oscillatoria* (7), *Schizothrix* (1), *Microcoleus* (2), *Phormidium* (9) and *Lyngbya* (08).

Keywords: Filamentous cyanobacteria, Karsa-Pohregaon barrage, Renapur, Maharashtra.

Introduction:

The diversity of blue green algae in abroad and in India has beendone extensively by many research workers.Reviews of literature reveals that, in Maharashtra tremendous work has been done on soil algal taxonomy by various workers. Gonzalves and Gangla (1949), Sardeshpande and Goyal (1981), Kolte and Goyal (1985) have been studied from the Vidarbha region. Marathe (1960-1969), Kottawar and David (1983), Mahajan and Mahajan (1989), Bhoge and Ragothaman (2003) studied from North Maharashtra region. In Marathwada region of Maharashtra except few reports (Shirode, 1981; Chaporkar and Gangawane, 1984) very rare attention has been paid towards soil algae, therefore to fulfill this lacuna, it has been decided to work on soil algae in the Marathwada region of Maharashtra.

Materials and Methods:

The Karsa-Pohregaon barrage is constructed on Manjara River at Pohregaon village in 2008, creating10.67 Mcum storage and 1990 Hector irrigation potential and also benefitted for the 9 villages from the Latur Tehsil and 7 villages from Renapur Tehsil to meet the requirement

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of drinking water and domestic purposes. The Pohregaon village is located located in Renapur Tehsil of Latur district in the Marathwada region of Maharashtra. It is situated 15km away from sub-district headquarters Renapur and 10km away from district headquarter Latur.

The soil samples were collected at morning between 8am to 10am from up to 6 inch depth from soil surface of dam area in the month of April (2017).The soil samples were placed in petriplates containing blotter paper and distilled water in laboratory conditions. The algae grow on blotter paper were used for morphological studies and preserved for the further investigations. The identifications of taxa were made by using Monograph by Desikachary (1959),Gomont (1892), Geitler (1932), Tiffany and Britton (1952), Prescott (1952) and available literature and research papers.

Systematic Enumeration:

Oscillatoria Vaucher, 1803

***Oscillatoria animalis* Agardh ex Gomont:**

Gomont 1892a, P.227, P1.7, F. 13; Geitler 1932, P.978, F. 603 a; Tiffany and Britton 1952, P. 346, P1.93, F. 1079, Desikachary 1959, P. 239, P1. 40, F. 14.

Thallus blue-green; trichomes straight, curved or bent at the ends, slightly constricted at the cross walls, not granulated at the cross walls, briefly attenuated at the ends; cells slightly shorter than broad, seldom longer, 3-4.8µ in diameter 2-4.5µ long; end cell conical, without a cap or a calyptra.

***Oscillatoria amphibia* Ag. Ex Gomont:**

Prescott 1951, P. 485, P1. 109, F.6; Desikachary 1959, P. 229. P1. 37, F. 6

Thallus blue-green; trichomes straight or coiled, not constricted at the cross walls, not attenuated at the ends; cells 2 times longer than broad. 2-2.5µ in diameter, 3.8-5.5µ long, with two granules at the septa; end cell rounded, without a cap or calyptra.

***Oscillatoria chalybea* (Mertens) Gomont:**

Gomont 1892a, P. 232, P1.7, F. 19; Geitler 1932.P. 956. F. 608 b; Prescott 1951, P. 486, P1. 109, F. 8,9; Tiffany and Britton 1952. P. 344, P1.93, F. 1071; Desikachary 1959, P. 218, P1.38, F. 3.

Thallus dark blue-green; trichomes usually bent at the ends, slightly constricted at the cross walls, very slightly tapering at the ends; cells shorter than broad, 5-7.2µ in diameter, 3-4.8µ long; end cell conical, without a cap or a calyptra.

***Oscillatoria margaritifera* (Kuetzing) Gomont:**

Desikachary 1959, P. 202, P1.42, F. 8.

Thallus blue-green to olive green; trichome straight, fragile, constricted at the cross walls, slightly attenuated at the ends; cells 1/3 – 1/7 as long as broad, 10.5-11.5µ in diameter, 2.8-4µ long, cross walls granulated; end cells capitate, with slightly convex calyptra.

***Oscillatoria martini* Fremy:**

Desikachary 1959, P. 216, P1.38, F.6.

Thallus blue-green; trichome loosely, irregulary spirally coiled, not constricted at the cross-walls, not granulated at the cros walls, attenuated at the ends, ends slightly curved; cells ½ - 1/3 as long as broad; 3.2-4.5µ in diameter, 1.5-2.5µ ling; end cell with flat, convex, distinctly thick, broad outer membrane.

***Oscillatoria princeps*Vaucher ex Gomont:**

Gomont 1892a, P. 206, P1 6, F.9; Geitler 1932, P. 947, F. 598a, 601c-g; Prescott 1951, P. 489, P1.110, F. 1; Desikachary 1959, P. 210, P1.37, F. 1, 10, 11, 13, 14.

Thallus blue-green; trichomes long, curved, not constrited at the cross walls, slightly tapering at the ends; cells (much shorter than broad) ¼ as long as broad, 15-16.5µ in diameter, 2.2-4.2µ long; not granulated at the cross walls, end cells capitate without a calyptra.

***Oscilatoria subbrevis* Schmidle:**

Geitler 1932, P. 946, F 601 b; Prescott 1951, P. 491, Pl. 107, F. 23; Desikachary 1959, P. 207, Pl. 37, F. 2, Pl. 40, F. 1.

Trichomes blue-green, single, nearly straight, not constricted at the cross walls, not attenuated at the ends; cells 6.5-7.2µ in diameter, 1.5-2µ long; not granulated at the cross walls; end cell rounded, without a cap or a calyptra.

***Schizothrix* Kuetzing, 1843.**

***Schizothrix friesii* (Agardh) Gomont:**

Geitler 1932, P. 1076, F. 685, 686; Prescott 1951, P. 507, Pl. 114, F. 5; Desikachary 1959, P. 328, Pl. 57, F. 1,6.

Thallus blue-green; filaments in lower parts contorted, above rarely straight; sheath hyaline, lamellated, acuminate at the ends, coloured violet by chlor-zinc-iodide, with few trichomes or single trichomes; trichomes distinctly constricted at the cross walls; cells nearly quadratic or 2 times as long as broad, 2.5-3µ in diameter, 3.8-6.2µ long; end cell obtuse, conical.

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***Microcoleus* Desmazieres, 1823**

***Microcleus lacustris* (Rabenh.) Farlow:**

Gomont 1892, P.359; Geitler 1932, P.1142, F.749, 750a; Prescott 1951, P.505, Pl.113, F.6; Desikachary 1959, P.345, Pl.60, F.4,5.

Thallus blue-green; filaments 32-36µ in diameter, contorted, seldom branched; sheath colourless, slimy, not coloured violet by chlor-zinc-iodide; trichomes distinctly constricted of the cross walls, cells 4-5.2µ in diameter, 6.5-8.5µ long, slightly attenuated at the ends; not granulated at the cross walls; end cell more or less rounded, conical, without a cap or a calyptra.

***Microcoleus vaginatus* (Vaucher) Gomont:**

(Geitler 1932, P.1136, F.741; Prescott 1951.P.506, Pl.131, F.2; Tiffany and Britton 1952, P.352, Pl.96, F.1112; Desikachary 1959, P.343, Pl.56, F.3.

Thallus dark green; filaments 25-27.5µ in diameter, sometimes sparsely branched, sometimes coiled; sheath hyaline, watery, uneven not coloured by chlor-zinc-iodide, often agglutinated with one another; trichomes blue-green or dirty green, not constricted at the cross walls, aften granulated at the cross walls, attenuated at the ends; cells subquadrate or ½-2 times as long as broad, 3.2-4.8 µ in diameter, 2-4.7 µ long; end cell capitate, with a flat, conical calyptra.

***Phormidium Kuetzing*, 1843**

***Phormidium ambiguum* Gomont:**

Gomont 1892 a, P. 178, Pl. 5, F. 10; Geitler 1932, P. 1015, F. 647 e; Prescott 1951, P. 493, Pl. 111, F. 1; Tiffany and Britton 1952, P. 350, Pl. 95, F. 1104; Desikachary 1959, P. 266, Pl. 44, F. 16, Pl. 45, F. 5-8.

Thallus blue-green; filaments flexuous, entangled, 5.4-6µ in diameter; sheath thin, firm, coloured violet by chlor-zinc-iodide; trichomes slightly constricted at the cross walls, not attenuated at the ends; cells shorter than broad, 4.5-4.8µ in diameter 1.5-2.5µ long; end cell rounded, without a cap or a calyptra.

***Phormidium anomala* Rao, C.B.:**

Desikachary 1959, P. 266, Pl. 45, F. 11-13.

Thallus expanded, soft, mucilaginous, dark blue green; filaments 9.5-10.1µ in diameter; sheath, thin, hyaline, not stained by chlor-zinc-iodide; persistant or dissolved; trichomes not constricted at the cross walls; cells disc shaped, much broader than long, 7-7.5µ in diameter, 1-1.2µ long; end cells bluntly rounded, without a cap or a calyptra.

***Phormidium corium* (Agardh) Gomont:**

Gomont 1951, P. 494; Desikachary 1959, P. 269. Pl. 44, F. 10-11.

Thallus expanded, membranous, leathery, brownish green; filaments long, more or less flexuous, entangled, 5.8-6.5µ in diameter; sheath thin, gelatinizing, coloured violet by chlor-zinc-iodide; trichomes not constricted at the cross walls, briefly attenuated at the ends; cells nearly quadrate, up to twice as long as broad, 4.5-4.8µ in diameter, 5-8.5µ long; not granulated at the cross walls; end cell obtuse, conical, without a cap or a calyptra.

***Phormidium calcicola* Gardner:**

Geitler 1932, P. 1013, G. 646 a; Desikachary 1959, P. 267, Pl. 43, F. 4,5.

Thallus blue-green, thick, firm, entangled; sheath thick, colourless, unlamellated; trichomes not constricted at the cross walls, not attenuated at the ends; cells slightly longer than broad, 6-6.5µ in diameter, 6.2-7.5µ long; not granulated at the cross walls; ends cell truncated, rounded, with a thickened outer membrane.

***Phormidium jenkelianum* Schmid G.:**

Geitler 1932, P. 1001, F. 638; Desikachary 1951, P. 255, Pl. 54, F. 7.

Thallus brownish black, slimy, filaments 3.5-3.8µ in diameter, flexuous, not attenuated at the ends; not coloured violet by chlor-zinc-iodide; trichomes brownish blue green, distinctly constricted at the cross walls, not granulated at the cross walls; cells ½ as long as broad, 3-3.8µ in diameter, 1.5-1.8µ long; end cell rounded, truncated, without a cap or a calyptra.

***Phormidium microtomum* Skuja:**

Desikachary 1959, P. 257, Pl. 43, F. 16,17.

Thallus expanded, lamellose, light blue-green; filaments more or less straight, 6.7-7.5µ in diameter; sheath thin, colourless later diffluent; trichome end briefly attenuated, not constricted at the cross walls; cells 5.2-6.5µ in diameter, 1-1.5µ long; end cell rounded with a hyaline calyptra.

***Phormidium molle* (Kuetzing) Gomont:**

Geitler 1932, P. 1000; Desikachary 1959, P. 255, Pl. 59, F. 8.

Thallus light blue-green, thin; sheath more or less diffluent, colourless, not coloured by chlor-zinc-iodide; trichomes 2.5-3µ in diameter, nearly straight, distinctly constricted at the cross walls, not attenuated at the ends, not granulated at the cross walls; cells quadrate, to longer than broad, 2.2-2.5µ in diameter, 4.8-6µ long; end cell rounded, without a cap or a calyptra.

***Phormidium pachydermaticum* Fremy:**

Geitler 1932, P. 1014, F. 648 b-e; Desikachary 1959, P. 267, Pl. 43, F. 8-10.

Thallus blue-green; filaments 6-6.5µ in diameter; sheath thick, lamellated, trichomes not constricted at the cross walls, not granulated at the cross walls, not attenuated at the ends; cells

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not quadrate, ½ as long as broad, 4.8-5.1µ diameter, 1.8-2.2µ long; end cell slightly convex or obtuse, conical, with a thickened membra

***Phormidium retzii* (Agardh) Gomont:**

Geitler 1932, P. 1012, F. 647; Desikachary 1959, P. 268, Pl. 44, F. 13-15; Prescott 1951, P. 495, Pl. 111, F. 6.

Thallus blue-green; filaments more or less straight, 6-6.8µ in diameter, sheath thin, diffluent, trichomes constricted at the cross walls, not granulated at the cross walls, not attenuated at the ends; cells 5-5.2µ in diameter, 4.5-5.2µ long; end cell rounded, without a cap or a calyptra.

***Lyngbya* Agardh, 1824**

***Lyngbya aestuarii* Liebm.exGomont:**

Geitler 1932, P. 1052, F. 666; Prescott 1951, P. 499, Pl. 111, F. 8; Desikachary 1959, P. 305, Pl. 52, F.8.

Thallus blue-green; filaments nearly straight, 13-13.5µ in diameter; sheath thin, lamellated, not coloured violetby chlor-zinc-iodide, trichomes not constricted at the cross walls, cross walls often granulated; cells 10.5-12.8µ in diameter, 2.5-3.5µ long; end cell flat with thickened membrane, slightly attenuated.

***Lyngbya birgei* Smith, G.M.:**

Geitler 1932, P. 1048, F. 663; Desikachary 1959, P. 296, Pl. 50, F. 7,8.

Filaments blue-green, straight, 16.5-17.2µ in diameter; sheath firm, colourless, unlamellated, seldom lamellated; trichomes not constricted at the cross walls, not attenuated at the ends; cells shorter than broad, 15-16µ in diameter, 2-3µ long, gas vacuoles not observed.

***Lyngbya dendrobia* Bruhlet Biswas:**

Geitler 1932, P. 1051; Desikachary 1959, P. 302, Pl. 50, F. 3,10, Pl. 55, F. 2-4.

Thallus blue green, more or less expanded, compact, thin; filaments long, flexible, more or less straight 7-7.5µ in diameter; sheath thin, smooth, hyaline; trichomes vary slightly constricted at the cross walls, uniformly, densely granular; cells 2 times shorter than broad (as broad as long), 6-6.8µ in diameter 2.5-3.2µ long; end cell rounded, without a cap or a calyptra.

***Lyngbya lagerheimii* (Moebius) Gomont:**

Geitler 1932, P. 1044, F. 661 g; Prescott 1951, P. 501, Pl. 112, F. 9; Desikachary 1959, P. 290, Pl. 48, F. 6, Pl. 53, F.2.

Filaments blue-green, 2.5-3µ in diameter, single or entangled with one another, irregularly spirally coiled; sheath thin, hyaline; trichomes not constricted at the cross walls, not

granulated at the cross walls, not attenuated at the ends; cells longer, end cell rounded, without a cap or a calyptra.

***Lyngbya major* Meneghini ex Gomont:**

Geitler 1932, P. 1066, F. 679 a; Prescott 1951, P. 502, Pl. 112, F. 10; Desikachary 1959, P. 320, Pl. 52, F. 11.

Filaments dark blue-green, long, straight, 15-16.5µ in diameter; sheath thick, colourless, lamellated, not coloured violet by chlor-zinc-iodide; trichomes not constricted at the cross walls, granulated at the cross walls, not attenuated at the ends; cells 1/4-1/8 as long as broad, 10-12.2µ in diameter, 2.5-3.2µ long; end cell rounded, with slightly thickened membrane.

***Lyngbya majuscula* Harvey ex Gomont:**

Geitler 1932, P. 1060, F. 672 c,d; Desikachary 1959, P. 313, Pl. 48, F. 7, Pl. 49, F. 12, Pl. 52, F. 10.

Thallus blue-green, expanded; filaments very long, curved or seldom, 14-15.1µ in diameter; sheath colourless, lamellated, colourless lamellated, not coloured violet by chlor-zinc-iodide; trichomes blue-green; not constricted at the cross walls, not attenuated at the ends, cells shorter than broad, 1/6-1/5 times as long as broad 11.7-12.5µ diameter, 2.8-3.5µ long; cross walls not granulated, end cell rotund, without a cap or a calyptra.

***Lyngbya semiplena* (C. Agardh) J. Agardh ex Gomont:**

Gomont 1892 a, P. 138, Pl. 3, F. 7-11; Geitler 1932, P. 1061, F. 672 a; Desikachary 1959, P. 315, Pl. 49, F. 8, Pl. 52, F. 7.

Thallus caespitose, dark yellowish-green; filaments 7.5-8µ in diameter, entangled, curved or straight; sheath hyaline, thick, unlamellated, not coloured violet by chlor-zinc-iodide; trichomes not constricted at the cross walls, often granulated at the cross walls, slightly attenuated at the ends; cells 1/3-1/2 times as long as broad, 6.2-6.5µ in diameter, 2.5-4.2µ long; end cell capitate, without a calyptra.

***Lyngbya spiralis* Geitler:**

Geitler 1932, P. 1042, F. 659; Desikachary 1959, P. 289, Pl. 48, F. 1.

Thallus blue green; filaments 8.5-9.4µ in diameter; spirals 6.8-9.4µ broad, the distance between two consecutive spirals 25.2-32.5µ; sheath hyaline, firm, not lamellated, not coloured violet by chlor-zinc-iodide; trichomes not constricted at the cross walls, not attenuated at the ends; cells shorter than broad, ½ times as long as broad, 6-6.5µ diameter, 2.5-3.2µ long; end cell broadly rounded, without a cap or a calyptra.

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Conclusion:

The present investigation was carried out during April 2017 to March 2018 to study the cynobacterial diversity from the soils of barrage in Renapur tehsil of Latur district in the Marathwada region of Maharashtra. A total of 82 taxa under 47 genera belonged to blue greens were encountered. The present paper deals with the systematic enumeration of only filamentous cyanobacterial forms like *Oscillatoria* (7), *Schizothrix* (1), *Microcoleus* (2), *Phormidium* (9) and *Lyngbya* (08),the results are agreed with Ashtekar,(1979c),Bhogeet.al (2007),Chaporkar,(1984),Kolte,(1985).

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MANAGEMENT OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* IN VEGETABLES

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Introduction:

Root-knot nematodes (Genus: *Meloidogyne*, Greek word means melon, apple or gourd-shaped female) are sedentary endoparasites of diverse crops. Root-knot nematodes are one of the five pathogens affecting world food production. Root-knot nematodes are a growing cause of concern for vegetable growers in India, especially in the production of crops in protected cultivation where one crop is grown repeatedly. Root-knot nematodes are the most frequently observed plant parasite species in the vegetable field (Koenning *et al.,* 1999). Damage of root-knot nematodes can be identified by the presence of galls on plant roots, galls are formed as a result of physiological disturbances in the root tissues caused by the trophic interactions of female nematodes, and identification of particular *Meloidogyne* spp. requires taxonomic analysis which is not feasible for farmers.

In India, the infestation of root-knot nematode was observed first time by Barber (1901) from a tea plantation in Kerala. Globally, there are 101 described species of *Meloidogyne*. In India 14 species of root-knot nematodes are recorded, among them, *M. incognita* (Kofoid and White, 1919), *M. javanica* (Treub, 1885) are widely distributed.

Bidhan Chandra Krishi Viswavidyalaya, Kalyani Centre collected 198 samples from different states and different agroclimatic regions, out of 198, 157 samples have shown infection, infection of *M. incognita* was found in 86%, of samples, followed by *M. javanica* in 26% samples, *M. arenaria* in 4% and *M. indica* in 2% of samples.

Root knot nematodes can cause significant loss in crops depending on genus, crop species and population, losses up to 30 % recorded in highly susceptible vegetables crops, brinjal, tomato and melon by Sikora and Fernandez (2005), in protected cultivation, crop loss due to nematodes can be up to 60 %. In India, an average loss of 21068.73 million rupees is estimated due to plant-parasitic nematodes (Rao *et al.,* 2015).

Table 1: Status and distribution of root knot nematodes races (Khan *et al.*, 2014)

***Meloidogyne* Species**	**Races**	**Distribution in different States/Union Territory**
M. incognita	1	India
	2	Andhra Pradesh, Assam, Bihar, Gujarat,Haryana, Himachal Pradesh, Karnataka, Kerala, Maharashtra, Mizoram, Odisha, Punjab, Tamil Nadu, Uttar Pradesh, West Bengal
	3	Assam, Gujarat, Haryana, Karnataka, Maharashtra, Puducheri, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal
	4	Haryana, Himachal Pradesh, Uttar Pradesh
	5	Haryana, Maharashtra, Tamil Nadu, Tripura
	6	Manipur
M. javanica	1	Haryana
	2	Uttar Pradesh, West Bengal
	3	Andhra Pradesh
	4	Andaman and Nicobar Islands, Gujarat
	5	Uttar Pradesh, West Bengal
	6	Haryana
M. arenaria	1	Haryana, Uttar Pradesh

Table 2: Economic loss and symptoms of root knot nematode infestation (Gowda *et al.*, 2017)

Family	**Crops**	**Loss**	**Symptoms**
Solanaceous	Tomato	11-35%	● Infestation in patches, ● Chlorosis, yellowing, wilting and stunt growth
	Brinjal	10-42%	
	Chilli	8-23%	
Cucurbitaceous	Cucumber	6-18%	● Infestation in patches ● Stunted growth ● Reduced vine length. ● Smaller and yellowish leaves ● Day time wilting in broad leaves cucurbits
	Bottle gourd	21-23%	
	Snake gourd	17%	
	Bitter gourd	13-14%	
	Pumpkin	13%	
Mallow	Okra	10-29%	● Stunted growth ● Poor emergence of seedlings ● Yellowing ● Foliage starts turning yellow from older leaves
Root crops	Carrot	18.20%	● Forking, distorted or stunted taproot ● Stubbing and fasciculation (bunching) of roots. ● Large conspicuous galls on feeder roots.
	Radish	18.20%	

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According to Trudgill and Blok, 2001 *M. incognita* is the single most crop-damaging pathogen causing $100 billion annual economic loss globally. High temperature, humidity, use of high agriculture input like fertilizers and plant growth promoters and repeated cultivation of crops increased the nematode build in protected cultivation, 10-30-fold, and more rapid build than in open field cultivation.

The root excludes from root-knot infected plants stimulate the entry of other soil born pathogens and increase the problems further leading to disease complex.

Table 3: Disease complex associated with root-knot nematodes

Disease Complexes	**Root knot Nematode species**	**Associated Pathogenic species**	**Vegetable Crops**	**References**
Damping off	*M. incognita*	*Rhizoctonia solani*	Tomato	Arya and Saxena, (1999)
Damping- off	*M. javanica*	*Pythium debaryanum*	Tomato	Ram Nath *et al.*, 1984
Bacterial wilt	*M. incognita*	*Ralstonia (Pseudomonas) Solanacearum*	Tomato	Haider *et al.*, (1987)
Fusarium wilt	*M. incognita*	*Fusarium oxysporum* f. sp. *lycopersici*	Tomato	Akram and Khan, 2006
Collar rot	*M. incognita*	*Sclerotium rolfsii*	Brinjal	Goswami et al., (1970)
Wilt	*M. incognita*	*Fusarium oxysporum* f. sp. *conglutinans*	Cauliflower	Pathak and Keshari (2004)
Powdery mildew	*M. javanica*	*Sphaerotheca fuliginea*	Cucumber	Khan and Pasha (1988)

Nematode infestation is more severe in polyhouse condition as it provides optimal condition (moisture level below 40-60%) of field capacity for survival and locomotion. The use of drip irrigation in protected cultivation provides optimal moisture around the root zone which facilitates rapid movement of nematodes. Nematode requires a thin layer of water to move. The optimum temperature for nematode multiplication is 25-35° C. In North India, the night temperature in open field condition is less, inside the polyhouse, night temperature is more which supports its development. Nematodes need some suitable host to continue their life cycle from

one generation to another, in polyhouse, one crop is grown over and over and the scope of crop rotation with the non-host crop is very limited, the farmer has already invested in polyhouse so leaving the polyhouse fallow or cultivation of low-value non-host crop can cause more loss than the damage caused by nematodes. Sandy and sandy loam soil have 50 % more sand, which makes movement of infective juvenile and root penetration easy, the damage of root-knot nematode is more severe in sandy soil than clay soil.

This chapter analyses the various technologies for the protection of root-knot nematodes *Meloidogyne* spp. in vegetables. Some practices are partially effective in the control of nematodes and the combination of different practices in a systematic approach provides a holistic solution to farmers. Nematicides are highly toxic to both human and the environment (Abawi and Widmer, 2000) and their use is restricted and progressively banned for use (methyl bromide is banned in developed countries). Thus, an integrated approach for long term management of nematode is required.

Prevention of nematode introduction in the field:

1. Selection of healthy seed material

Root-knot nematodes are introduced from one field to other by contaminated seeds, plant material, soil, irrigation water and other human activities like use of agriculture equipments from infected field to healthy field. Seeds of crop must be purchased from reliable dealers. For the polyhouse, it is advisable to grow seedlings in soil less media, in clean and sterilized trays. Special care should be taken by growing crop to avoid nematode infestation.

Cleaning of all the agriculture equipment and tools is recommended for use to prevent transport of nematode with soil (Mateille *et al.*, 2005; Djian-Caporalino *et al.*, 2009). Plant material also introduce the nematodes from one field to other, by heating, or spraying and coating with natural nematicide solution, or use of *in vitro* grown healthy plant material, their spread can be easily controlled (Bridge, 1996). It is also essential to check that seeds are free of nematodes and properly treated before sowing in the field.

2. Construction of new polyhouse

If a farmer is constructing a new polyhouse in a field that has a long history of vegetables, should be avoided as the field is most likely to be already infected with root-knot nematodes which damage vegetable crops, though field which is used for cereal crops may be better as it usually may not harbour nematodes that attack polyhouse crops. It is also recommended for soil testing for nematode infestation before constructing a new polyhouse.

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3. Prevention of secondary infestation

Once the nematodes are introduced in the field, they move from one part to other parts of the field by different means and it is important to control further spread of nematode from nematode infested field.

4. Irrigation management

Nematodes move from one to another field easily in wet soil and it offers the best condition to achieve their life cycle, soil moisture also enhance the egg hatching. Optimal irrigation can reduce nematode infestation.

5. Plant residue cleaning

Removal of contaminated crop plant and roots reduces the multiplication of nematodes after harvest, Bridge (1996) suggested uprooting and sun exposing roots to reduce nematode infestation.

6. Weed management

A wide range of weeds including *Amaranthus* spp. are also the alternate host for nematodes. Removing these weeds during fallow, the nematode population decreases. In the vegetable cropping system, weeds such as *Chenopodium album, Solanum nigrum, Tithonia rotundifolia* and other unknown weeds are known to act as excellent hosts for the perpetuation of root-knot nematodes (Khan *et al*. 2014).

7. Escape cropping

Low temperature increases the nematode life cycle and reduces reproduction and hatching. Planting of crops in winter when soil temperature is low, can manage the nematode population as nematode cannot be much active at low temperature.

8. Antagonistic crops

Some crops have chemicals that are harmful to nematodes, for example, marigold has α – terthinyl and bithinyl compounds which kills the nematodes, allyl isothiocyanate in mustard, also kills nematodes, planting these crop before the main crop also reduces the nematode infestation. Plants that release certain root exudates in the rhizosphere having nematicidal values, when inter-cropped with the susceptible crop or grown as cover crops such as African marigold, mustard, sesame and asparagus (*Asparagus officinalis*) can also help in reducing the population of root-knot nematodes (Gaur, 1975, Haque and Gaur, 1985).

9. Crop rotation

Crop rotation with the non-host crop is helpful in reducing inoculum level in the field. In vegetables, root-knot nematodes usually survive in the soil either in the form of eggs or second-

stage juveniles. In the absence of a host plant, the populations of root-knot nematode juveniles have been observed to decline by 75 % in 3-4 months due to starvation, desiccation, heat etc., if weeds or other hosts are not available. Crop rotation with non-host crop like sesame, mustard, wheat, maize suppresses the nematode population (Haque and Gaur, 1985, Siddiqui and Saxena, 1987). In vegetable crops, rotation with vegetable crops can increase the nematode infestation, so crop rotation with a non-host crop like okra-cowpea-cabbage or okra-cucumber-mustard is recommended to reduce infestation. In potato, inter-cropping with onion and maize has been found to reduce galling due to *M. incognita*. The okra-cowpea-cabbage and okra-cucumber-mustard sequences showed a maximum suppressive effect on *M. incognita* in West Bengal conditions (Chandra and Khan, 2011).

10. Summer ploughing

Deep summer ploughing exposes nematode and infected soil tissues to solar heat and dehydration and reduces nematode infestation. Normal (10 cm) and deep ploughing (20 cm) in June followed by a fallow period of 2 months significantly reduces the nematode infestation. The efficiency of summer ploughing is further improved by solarisation to harness the benefits of solar energy which helps in trapping and retaining more heat under polythene mulching than the direct exposure alone (Gaur and Perry, 1991).

11. Trap crops

Traps crops are those which are highly susceptible to nematode infestation. These crops are allowed to grow over a period to invade and develop but do not support the nematode for the entire life cycle. By carefully planning, the susceptible trap crop can be planted first and then removed and burnt so that the main crop can escape the nematode infestation. *Crotalaria spectabilis* is a commonly used crop but its success depends on the time of planting and crop destruction.

12. Cover crop

Crop cover supplies the organic amendments, improve soil structure, protect the crop of soil erosion, suppress nematodes, weeds, insects, and improve the soil structure. These crops are planted or incorporated between the cultivation of an annual cash crop. Cover crops like *Crotalaria*, castor bean, velvet bean, jack bean, sorghum-Sudan, castor etc. are non-host crops, as nematode can move only a very short duration, some population of nematodes may starve, and so, nematode infestation can be reduced. They can also be incorporated as green manure, which releases heamatotoxin compounds after decomposition and reduces the nematode population.

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Table 4: Nematicidal components of cover crops used for nematode management

Sr. No.	Nematode suppressive crops	Nematicidal compounds
1	Marigold	Polythienyl-alpha-terthienyl
2	Crotalaria spp.	Monocrotoline, Pyrrolizidine
3	Secale cereale (Rye)	Butyric acid and Hydroxamic Acid
4	Sudan grass	Cyanoglycoside dhurrin
5	Castor	Ricin
6	Brassicas (Rape seed and Mustard)	Isothiocyanates
7	Velvet bean	1-tricontanol, triacontanyl tetracosanate
8	Sesame	Acetic acid

13. Soil solarization

It is a method of placing plastic sheets on moist soil during a period of high ambient temperature. These sheets trap the sun's radiant energy and heat the upper layer, thus kills many soil-borne pathogens including root-knot nematodes. For the soil solarization, soil beds of smooth soil are prepared and irrigated with 70 % of field capacity and moist up to a depth of 24 inches. The plastic sheets should be left for 4 to 6 weeks to allow the soil to heat the greatest depth possible. It can be done with a thin transparent polythene sheet (50-100 µm).

14. Removal and destruction of infected plants

Early identification and removal of infected plants can reduce the nematode infestation. After harvesting, the stubble of infected crops needs to be removed and burnt as they can serve as an inoculum for next season.

15. Genetics based method

It includes the use of genetic and biotechnical method for control of root-knot nematodes in the field.

a. Host plant resistance:

It includes evaluation of nematicide resistant germplasms/genotypes or wild relatives of different vegetable crops and development of nematode-resistant plant varieties. Mi gene from wild tomato species *Solanum peruvianum* is resistant to *M. incognita*, *M. javanica* and *M. arenaria*. It is introduced in tomato crops using isozyme marker, Aps-1 and DNA markers, Rex-1.

Table 5: Nematode resistant varieties of different crops

Sr. No.	Crop	Variety and lines
1	Tomato	SL-120, PNR-7, Hisar Lalit, NT-3, NT12, Pusa Hybrid-2, Arka Vardana, Azad T-8, CO-3, Lalit, PT4716A, LE812, Hisar N1, Hisar N2, Patriot, SL-120
2	Chilli	Pusa Jwala , NP-46A, Mohini, Pusa Sadabahar, Guchheedar, PSL-3, PC-56, Surajmukhi, BSS-138, LCA-304, LCA-305, Hoe-808, CH-27, CA-960, G-4, Surajmukhi, ZCH-3025, BSBS-172, Pant Chilli-4, LCA-206, Roshni, Brahmpur, CH-1, Byadagi Kaddi
3	Brinjal	Black beauty, Banaras Giant, Rajendra Baigan, IC-90903, IC-127029, Rajendra BaiganII long, Pant Rituraj, IC- 122076, KS-224, IC-127040
4	Okra	Abelmoschus moschatus (two genotypes viz. IC-14O970-A, IC-203863) and Abelmoschus angulosus genotypes (IC- 470751, IC-203834, IC-203831, IC-203833, 1C-203863)
5	Bottle gourd	Samrat, Bogh-2, PSPL, Hoe-505,
6	Sponge gourd	Kashi Jyoti
7	Potato	Kufri Swarna
8	Carrot	Arka Suraj

b. Protease inhibitors

Protease (proteolytic enzymes or proteinases) are a group of enzymes that hydrolyse peptide bonds of proteins. It is classified into four groups, cysteine, serine, aspartyl and metal- based on the ability to hydrolyse various peptide bonds. Nematodes require proteases to digest protein into amino acids for protein synthesis, protease inhibitor block nematode proteases which are responsible for parasitism through host protease. cowpea trypsin inhibitor (CpTi) has the potential of controlling golden cyst nematode in potato. *Cicer arietinum* proteinase inhibitor (CaPI) treatment reduces root gall formation and egg mass production in tomato.

c. RNA interference (RNAi)

RNAi refers to sequence-specific and homology-dependent gene silencing through a complex mechanism in which double-stranded RNA (dsRNA) is recognized which leads to a chain of events resulting in the degradation of both the dsRNA and homologous RNA.

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16. Biological control

Biological control includes the use of fungal and bacterial parasite to control nematodes. These organisms feed or parasite the nematodes and release the secondary metabolites with nematicidal activity. Nematode suppressive soils are made by inoculation with effective antagonists at higher concentration to attain immediate control normally termed as inundation strategy as well as inoculation strategy in which long-term effects are achieved through colonization of nematode antagonists in soil.

a. Fungal antagonists

These soil-borne fungi include nematode-trapping fungi, egg parasites and toxin-producing fungi**.**

b. Nematode trapping fungi

Arthrobotrys spp. and *Monacrosporium* SPP. trap nematodes by constricting rings and adhesive nets respectively, it involves an association between lectin secreted by the fungus and a carbohydrate secreted by the nematode cuticle. In certain soil condition, it is effective against the control of *M. incognita* and *M. javanica* in vegetables.

c. Egg parasites

Paecilomyces lilacinus is commercially used as fungal bioagents. Sedentary stages of the nematode life cycle i.e., egg mass and obese females are vulnerable to fungal colonization or parasitisation. Fungal hyphae of fungi grow in the gelatinous matrix and penetrate individual hyphae through egg cuticle and then eggs are engulfed with the mycelial network. It can control *M. javanica* and *M. incognita* population in tomato, brinjal and other vegetable crops. *P. chlamydosporia* proved its potential egg parasitism in root-knot nematode, *M. incognita.*

d. Toxin producing fungi

Filamentous fungi *Trichoderma* spp. (*Trichoderma viride* and *T. harzianum*) are commercially used for control of root-knot nematodes in vegetable crops. These fungi directly parasitise on the eggs through increasing extracellular chitinase activity and induce systemic resistance in plants.

e. Antagonistic Bacteria

i. Spore forming bacteria

Pasteuria penetrans is gram-positive endospore-forming, obligate parasitic bacteria. It attaches its adhesive endospores on second-stage juvenile's cuticle and their proliferation inside nematode body by utilizing resources for its growth by synchronizing its life cycle with nematode development and causing sterility in females by disrupting nematode reproductive

system. Large scale mass production of this bacterium is difficult due to obligate parasitism which limits its commercial utilisation.

ii. Plant growth promoting rhizobacteria (PGPR)

PGPR have the potential for control of root-knot nematodes. They are present in the rhizosphere region of plant and compete for nutrient, antibiosis, plant growth promotion and induced systemic resistance in plant against nematodes. Many studies prove the potential of *Pseudomonas fluorescens* and *Bacillus* spp. Strains of *P. fluorescens* which produce an antibiotic DAPG (2, 4-Diacetyl phloroglucinol) is responsible for high nematicidal action.

Application of fungal and bacterial treatment:

- **Seed treatment**
  - Direct seeded crop such as okra, cucurbits seeds are treated with fungal and bacterial antagonists @ 10 g/kg seed.
  - *Pseudomonas fluorescens* and/or *Trichoderma harzianum* can be used as seed treatment or seed dressing agent.
- **Nursery treatment**
  - Antagonists (*Trichoderma harzianum, Paecilomyces lilacinus* or *Pseudomonas fluorescens*) are used for the treatment of transplanted vegetable seedlings of tomato, brinjal and chilli in nursery beds @ 50g/m^2 area.
- **Main field**:
  - Incidence of root-knot nematodes can be controlled by application of antagonists, *Paecilomyces lilacinus* or *Pseudomonas fluorescens* in field @ 10 kg/ha in combination with 1.5 ton of FYM/ha on the beds 5 to 10 days before sowing seeds. It should be covered with mulch with optimum moisture of 25 – 30 % for a period of 15 days and thoroughly mixed once a week for maximum multiplication and homogenous spread of micro-organisms.
  - In bitter gourd, seed treatment of *Pseudomonas fluorescens* and fungal bio-gent, *Trichoderma harzianum* at the rate of 10 g/kg seed and soil application of 10 kg/ha with 1.5 ton FYM reduces the root-knot incidence.
  - In Okra, seed treatment with *Pseudomonas fluorescens* 1% W.P. (2 x 108 cfu) @ 20g/kg seed with 5 tons of FYM and 2.5 kg of each *Paecilomyces lilacinus* (2 x 106 cfu/g) + *Pseudomonas fluorescens* (2 x 108 cfu/g) is found effective.
  - In brinjal, application of neem cake @ 1.5 t/ha 10 days before transplanting of seedlings and soil application of talc-based formulation of antagonists *Pseudomonas fluorescens* +

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Trichoderma harzianum @ 10 kg/ha along with 1.5 t/ha at the time of transplanting is found effective.

- In chilli, combined application of talc-based formulations such as *Trichoderma viride* (30g/10 m2), *Pochonia chlamydosporia* (20g/10 m2) and neem cake (0.15 kg/10 m2)
- Enrichment of Nematode antagonists 30 showed greater plant growth with a significant reduction of root-knot nematode (*M. incognita*).

Table 6: Commercially available bioagents, their manufacturer

Bioagents	**Trade names**	**Manufacturer**	**References**
Pseudomonas fluorescens 1% W.P. *Trichoderma harzianum* 1% W.P. *Trichoderma viride* 1.5% W.P. *Paecilomyces lilacinus* 1% W.P. *Pochonia chlamydosporia* 1% W. P		IIHR	https://www.iihr.res.in/ Rao *et al.*, 2015
Paecilomyces lilacinus 1% W.P.	PAECILO®	PAECILO® Agri Life Bio solutions for soils and Crops, Hyderabad	http://www.agrilife.in
Paecilomyces lilacinus 1% W.P.	MYSIS®	MYSIS® Varsha BioScience &Tech., Hyderabad	http://www.varshabioscience.com
Paecilomyces lilacinus 1% W.P.	BIONICONEMA®	BIONICONEMA® Nico Orgo Manure, Gujarat	http://www.neemnico.com/
Pseudomonas fluorescens 1% W.P.	POWER ALL	POWER ALL® Nico Orgo Manure, Gujarat	http://www.neemnico.com/
Paecilomyces lilacinus 1% W.P.	BioAce®	Indore Biotech Inputs and Research (P) Ltd, Indore	http://www.indobioagri.in
Paecilomyces lilacinus (Wettable Powder and Aqueous Suspension)	Nemator®	Biotech International, New Delhi	https://www.biotech-int.com
Paecilomyces lilacinus 1% W.P.	Nemastin®	Kans Biosys, Maharashtra	http://kanbiosys.com
Paecilomyces lilacinus	Jaiveek	Jaiveek food and fertilizers	http://www.jaiveekfertilizer.com/
Paecilomyces lilacinus	**Nematoz - P**	Utkarsh Agrochem Pvt ltd	https://utkarshagro.com/

- **On standing crop**
    - Drenching through drip irrigation of fungal formulation of *Trichoderma harzianum*, *T. viride* and *Paecilomyces lilacinus* and bacterial antagonists *Pseudomonas fluorescens* and *Bacillus subtilis* @ 0.5 % at regular interval of 30 days after sowing or transplanting can be used for nematode control.

Organic amendments increase the efficiency of root knot nematodes by suitable environment for their growth and development.

Organic amendments:

It is a traditional activity that increases soil physical condition and biological activity, improves soil fertility, and recycles nutrients. Organic amendments can be animal manures like poultry or cattle, green manure from crops and their residue and different plants parts like chopped leaves, leaves extract and industrial waste like oilseed cake. Organic amendments help crops in nematode management in three different ways.

- Simulate the microbiological activities like nematode antagonists, predators, and parasites in the soil during decomposition.
- Release of nematicidal components after decomposition.
- Increase in plant immunity power by improving plant vigour and tolerance and increase in soil capacity to hold nutrients.

Nematode management potential of organic amendments depends on the Carbon (C) to Nitrogen (N) Ratio. Amendments which have a ratio of 12 to 20 are highly suitable for crop. Efficiency of organic amendments depends on many factors -

- **Quantity**: Nematode population reduces as the quantity of organic amendment applied is increased, however after a certain point, it can cause phytotoxicity.
- **Number of application year**: Some trials have confirmed that nematicidal effects of amendments are cumulative and their efficiency increased when applied for the second and third year, e.g., Sudan grass as a cover crop control infestation *of M. hapla* in the second and third year.
- **Chemical characteristic of different product**: Brassica reduce nematode infestation as after decomposition it releases toxic components like isothiocyanates produced from glucosinolates which are toxic to nematodes. This process is also called as bio fumigation. Glucosinolates concentration in crop depends on the age of crop when chopped, soil type, moisture and temperature.

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- **Physiological stages of the incorporated plant tissues:** The concentration of nematotoxic compoundsin a crop depends on their age. In Sudan grass, the concentration of hydrogen cyanide due to dhurrin decomposition decreases as the crop mature.
- Soil infestation level and the nematode community structures.

Type of organic amendments

1) **Plant produce:** Crop and plant species of 57 families are identified which have nematicidal properties. The plant species release nematotoxic components through volatilization, exudation from roots, leaching from plants or residues, and decomposition of residues.

   Neem: Leaf, seed kernel, seed powders, seed extracts, oil and oilcake of neem is used as nematicide. Neem release components such as salanin, azadirachtin, nimbin, thionemone and various flavonoids. In neem, triterpene components in neem oil cake inhibit the nitrification process and increase available nitrogen for the same amount of fertilizer. Application of neem seed cake @ 0.5 kg/m^2 area followed by solarization in raised seed nursery with the mulching sheet and application of neem cake @ 1-2 t/ha. neem cake is required to manage root-knot nematode under field condition. Neem (*Azadirachta indica*) and Subabool (*Leucaena lucocephala*) leaves used as nursery soil treatment @ 50 q/ha in tomato gave better seedling growth and reduced galling (Jain and Bhatti, 1983; Jain *et al.*, 1988; Mojumder and Mishra, 1993)

   Castor: Application of Castor cake or Neem cake or Pongamia cake @ 500 g/m^2 followed by solarization of the nursery beds and leaving the nursery beds for 20 days can be used for the production of nematode free seedlings.

   Mustard: Application of *Trichoderma viride* at 0.5 kg/ha + mustard cake at 50 kg/ha, or *P. lilacinus* at 0.5 kg/ha + mustard cake at 50 kg/ha can reduce the nematode infestation in vegetables significantly.

2) **Animal manures**

   Goat manures: Development of healthy root system and aggregating ability of soil particles is high in goat manure, healthy root system requires elements such as phosphorus and potassium which are high in goat manure (P=0.7 %, K=1.9 %), sufficient supply of these elements help plants in healthy root development, which suppress the nematode population. Goat manures have Pellet like structure that helps in soil aggregation, which restrict the free movement of nematodes.

Chemical control:

Control of nematodes using chemical is a challenge as nematodes spend their lives confined to the soil or within plant roots, delivery of a chemical to the immediate surroundings of a nematode is difficult. The outer surface of nematodes is a poor biochemical target and is impermeable to many organic molecules. Nematode ingests the material only when feeding on plant parts so delivery of chemical through oral route is also difficult.

Judicious or need-based application of nematicides is recommended in the case of highly susceptible crops and high-value cash crops or for early protection of tender stages.

Carbamate group chemicals, Carbofuran 3G and Carbosulfan 25 EC are used for control of root-knot nematodes.

- Application of Carbofuran 3G @ 0.3 g a.i /m^2 area of nursery bed.
- Bare root treatment of seedlings with Carbosulfan 25 EC @ 2 ml/l during transplanting crops.
- Seed dressing of directly seeded crop like okra and cucurbits with Carbosulfan 25 DS @ 3 % a. i. (w/w) effectively manage root-knot nematode incidence in vegetable crops.
- Seed treatment with Carbosulfan (25 EC) at 0.1 % for overnight or root dipping 0.05 % for 6 hours in cucurbitaceous crops.
- Application of Carbofuran 3G @ 1 kg a.i/ha is recommended to nematode infested vegetable crops under field condition.

Some other pesticide companies have developed pesticide based on fluopyram, fluensulfone which have nematicidal properties.

Table 7: Commercially available chemicals for nematode control

Sr. No.	Brand name	Chemical	Manufacturer
1	NIMITZ	Fluensulfone 2 % Gr	Adama India
2	Velum prime	Fluopyrum 34.48% SC	Bayer
3	NEMATHORIN® 150EC	Fosthiazate	Syngenta

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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL INVESTIGATION OF LEAF EXTRACT OF *TRIDAX PROCUMBENS* L.

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Abstract

Medicinal plants have been used extensively against various diseases over a long period of time. *Tridax procumbens* L.is a medicinal plant which has various therapeutic values. It is usedfor the treatment of skin diseases, epilepsy, dysentery, and diarrhea, preventing hair loss etc.The present study deals with bioactivities of leaf extract of *Tridax procumbens* L. Ethanol extract from leaves of this plant shown antibacterial activity against Gram negative bacteria *E.coli* and Gram positive *S. aureus* which was done using disc diffusion method. The qualitative phytochemical analysis was also done which showed presence of substances like tannins, phenols, flavonoids, proteins etc. Ethanolic extract of leaves of *Tridax procumbens* L. also showed enhanced anticoagulant activity. These activities prove this plant as a good candidate for pharmaceutical industry.

Keywords: *Tridax procumbens* L., ethanol extract, phytochemical, anticoagulant, flavonoids.

Introduction:

Plants play vital role, as it is one of the most important component of biodiversity. Our universe composed of about 5, 00,000 species of plants (Sawant and Godghate, 2013). For centuries plants have been used for nutritional as well as medicinal purposes. Over the years, these herbal drugs have proven to be effective (Awe and Omejasda, 2003). Almost several drugs used today derived from natural sources. Many of plant extracts are mainly used in traditional medicines because they are readily accessible in rural areas and cheaper than modern medicines.

Several plants and their parts are mostly used for treatment of various diseases (Girish and Satish, 2008). Medicinal plants synthesize substances that are useful for maintenanceof humans and animals health. These substances called secondary metabolites which play important role in defense mechanism against various microbes and insects (Nirmaladevi *et al.,* 2008).

Tridax procumbens L. is a common weed found in India and many countries all over the world growing during rainy season is one such medicinal plant. It has been extensively used in medicine as anticoagulant, antifungal and insect repellent. Its leaf extracts were known to treat skin diseases, liver disorders besides gastritis and heart burn (Wani *et al.,* 2010). The plant has been established for treatment of wound healing, dysentery, epilepsy, hypertension, hepatotoxicity, hemorrhage and metabolic syndrome (Suseela *et al.,* 2002).*Tridax procumbens* L. was well known in traditional medicine as abundant wild herbal which can treat various diseases, such as bronchial catarrh,diarrhea, preventing hair loss. (Taddei *et al.,* 2000).

The aim of the present study was to prepare ethanolic extract of leaves of *Tridax procumbens*L. and to perform phytochemical screening ofthat extract .The extract was also studied for antibacterial activity against both gram positive and gram negative bacteria using disc diffusion method. The blood clotting activity was also checked of that extract.

Material and Methods:

Plant Material

The plants were collected from nearby farm of Chopda from Chopda tahsil of Jalgaon District of Maharashtra. The plant leaves were separated from plant and washed with water to removedirt. Further the leaves were shade dried for two weeks.

Preparation of Extract

Dried leaves were grounded to granular powder using mechanical grinder. The powdered sample was stored in air tight containers. 20 gm. of powdered sample and 300 ml of solvent was used.The powder was extracted with ethanol according to the maceration method andethanol extract was filtered by filter paper (Whatmann no.1). The filtrates obtained were combined and then evaporatedto dryness under reduced pressure (Phrompittayarat *et al.,* 2007; Sasidharan *et al.,* 2008). The extract was stored at 4^{0} C until further use for various evaluations.

Phytochemical screening

Ethanol extract of leaves of *Tridax procumbens* plant was testedfor qualitative phytochemical analysis using standard procedures (Trease and Evans, 1983; Sofowora, 1993; Hedge and Joshi, 2010). Tests were performed for carbohydrates, amino acids, proteins, steroids, anthocyanin, flavonoids, phenols, and tannins.

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Carbohydrates test (Benedict's test)

1 ml of extract was treated with 5 ml Benedict's reagent and boiled for few minutes; formation of red precipitate indicates the presence of carbohydrates.

Proteins (Xanthoproteic Test)

Extract was treated with few drops of conc.HNO_3; formation of yellow color indicates presence of proteins.

Amino acids (Ninhydrin Test)

3 ml of extract was treated with 3 ml of ninhydrin reagent and allowed to boil for few minutes; formation of blue color indicates the presence of amino acids.

Steroids

0.5 ml of extract was dissolved in 5ml of chloroform. To this mixture equal volume of conc. H_2SO_4 was added from the side of the test tubes. Upper layer at the surface appeared red and acidic layer showed yellow with green fluorescence indicating the presence of steroids.

Phenols (Ferric Chloride Test)

3 ml of extract was treated with few drops of alcoholic $FeCl_3$ solution; appearance of bluish black color indicates the presence of phenols.

Anthocyanin

2ml of extract was added to 2 ml of 2N HCL and NH_3 resulting in to the appearance of pink red color which turns in to blue violet indicating presence of anthocyanin.

Tannins

2 ml of extract was treated with few drops of $FeCl_3$ solution; appearance of green color indicates the presence of condensed tannins.

Flavonoids (Alkaline Reagent Test)

1 ml of extract was treated with 1 ml 10% NaOH solution resulting in to the formation of deep yellow color indicates presence of flavonoids.

Blood Coagulation activity

To detect the blood coagulation activity 1 drop of blood was mixed with the 1 drop of leaves extract with the concentration of 10% (W/V) prepared by fresh weight basis. The time required for blood clotting was observed and compared with time required for normal blood clotting (Kokate *et al.,* 1997).

Antibacterial activity

The antibacterial activity of *Tridax procumbens* was investigated on both Gram positive and Gram negative bacteria such as *Staphylococcus aureus* and *Escherichia coli*. The antibacterial activity of ethanol extract of plant leaves was evaluated using disc diffusion method

(Bauer *et al.,* 1966; Parekh and Chanda, 2006). The media (Muller Hinton Agar no.2) along with inoculum was poured into petriplate. The extract concentration 1mg/ml was used to evaluate the antibacterial activity. Disc was saturated with 100µl of test compound and placed on upper layer of seeded agar plates (Jhample *et al.,* 2015). Antibiotic gentamycin used as a positive control ata conc.of 100 µg/ml (Dhanabalan *et al.,* 2008; Krishnaswamy and Christina, 2015). The plates were incubated at 37^0 C for 24 hrs. Microbial growth was determined by measuring the diameter of zone of inhibition.

Results and Discussion:

Phytochemical screening

Table 1: Phytochemicals present in Ethanolic extract of *Tridax procumbens*

Sr. No.	Phytochemicals	Ethanol extract of leaves
1	Carbohydrate	+
2	Protein	+
3	Amino acid	+
4	Steroid	+
5	Phenol	+
6	Anthocyanin	+
7	Tannin	+
8	Flavonoid	+

+ = Present

Antibacterial activity

Table 2: Antimicrobial activity of Ethanolic extracts of *Tridax procumbens*

Organism	Zone of inhibition in mm	
	Ethanol leaves extract	Gentamycin
Escherichia coli	11	14
Staphylococcus aureus	9	15

The phytochemical analysis of leaves extract of *Tridax procumbens*plant was given in Table 1.Flavonoids are known to be synthesized by plants in response to microbial infection. Hence it should not be surprising that they have been found to be effective as antimicrobial substances against wide array of infectious agents (Jamine *et al.,* 2007). Tannins are also known as antimicrobial agents. They are water soluble polyphenols and participated proteins present in many plant foods. Tannin has been reported to prevent the development of microorganism by

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precipitating microbial protein. The growth of many fungi, yeasts, bacteria and viruses were inhibited by this compound. They have been reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically, tannin-containing plants are used to treat nonspecific diarrhea, inflammation of mouth, throat and slightly injured skins (Prasad *et al.*, 2008).

The result of antibacterial activity was given in Table 2. Ethanolic extract of leaves showed better antibacterial activity against *E.coli* than *S. aureus.*While gentamycin showed better activity against *S. aureus*than *E.coli* .The present result revealed that the extract of *Tridax procumbens* was effective against both gram positiveand gram negative bacteria used in the study. The time required for normal blood clotting of samplewas found to be 150 sec. while time required for that sample in presence of the extract was 120 sec. Hence it may be used as hemostatic agent.

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IN VITRO CLONAL PROPAGATION IN *CENTELLA ASIATICA*

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Abstract:

Clonal propagation aims at the production of disease free, true-to-type plant production in larger numbers, in quicker time. In *C. asiatica*, many reliable approaches have been developed for *in vitro* regeneration at commercial level. Effective regeneration of *C. asiatica* using pre-existing meristems is affected by many internal and external factors and *in vitro* conditions and has been comprehensively covered here. Specific requirements during clonal propagation, like the culture establishment, shoot bud multiplication, root induction and acclimatization are discussed here. There are some cost effective methods also. In *C. asiatica*, there are several reports which indicate rapid regeneration and multiplication through organogenesis or by the process of somatic embryogenesis. There are also reports which suggest increase in amount of active compound in tissue culture.

Keywords: *Centella asiatica, In vitro*, Organogenesis, Rooting, Shoot multiplication, Somatic Embryogenesis

Abbreviations: IAA- indole-3-acetic acid; IBA- indole-3-butyric acid; Kn- kinetin; NAA-α-naphthaleneacetic acid, PGR- plant growth regulator; TDZ- thidiazuron; 2,4-D- 2,4-Dichlorophenoxyacetic acid

Introduction:

Clonal propagation is a type of asexual reproduction where genetically similar copies of a plant are produced and the plant population derived from this process is known as clone. *In vitro* clonal propagation is known as micropropagation. Totipotency is the characteristic feature of a plant cells by which plant cell can produce a whole plant (Reinert and Backs, 1968; Verdeil *et al.*, 2007). There are five stages of micropropagation (George and Debergh, 2008): First stage is called Stage 0— i.e. pre-treatments stage. Second stage is Stage 1— also known as establishment of culture; this stage is followed by Stage 2, where shoot bud multiplication occurs. The next stage after the shoot bud multiplication is Stage 3 — i.e. root induction; and the final stage is

Stage 4 or acclimatization stage. In Stage 0, selection and pre-treatments of mother plants are carried out successfully (George and Debergh, 2008). For clonal propagation, stock plants need to be pre-treated. Some pre-treatments can be necessary either for reducing the contamination of the stock plant in order to be able to have successful surface sterilization of explants or for enabling or improving the growth of explants in subsequent *in vitro* conditions. In Stage 1 explants are surface sterilized so that they can survive and grow under controlled conditions. After successful initiation of culture (i.e. culture without any contamination) shoot bud multiplication is achieved during Stage 2. Shoot bud multiplication depends on various factors, such as plant species, cultivar or genotype; organic and inorganic compounds, plant growth regulator (PGR) content and some culture conditions such as light, temperature, vessel humidity etc. This stage involves the development of *in vitro* shoot bud. During Stage 3 shoots originated from Stage 2 are then transferred for root induction either under *in vitro* or under *ex vitro* conditions. Successful rooting also depends on several factors. Transfer of tissue culture grown plantlets to the natural environment occurs during Stage 4. This process is a crucial step for commercial use of micropropagation. Micropropagated plants are not totally dependent on photosynthesis. In culture tube or any culture vessels humidity is very high that results in some morphological and physiological modifications of plants which results rapid water loss of plants when transferred to external conditions. During acclimatization process humidity is decreased step-by-step and concomitant increase in light intensity.

Centella asiatica L., a member of Apiaceae, commonly known in India as 'Indian Pennywort' or 'Mandookaparni'. It is a perennial herb, prostrate in nature, slightly aromatic stoloniferous, stem glabrous, petiole long with fleshy leaves, roots present at node. *C. asiatica* grows in India up to an altitude of 600 m above sea level (Patra *et al.,* 1998). In Indian medicine, *Centella* is used as reputed nerve tonic and also used for curing of asthma, bronchitis, dropsy, elephantiasis, gastric catarrh, kidney troubles, leprosy, leucorrhoea, skin disease and urethritis (Kakkar, 1988) with antibacterial, antifeedant, antifilarial, antileprotic, antistress, antituberculosis activities and wound-healing properties (Chakraborty *et al.,* 1996; Srivastava *et al.,* 1997). Leaves of *Centella* are rich in carotenoids, vitamins B and C. The plant also shows good therapeutic effects on peptic ulcers. It is reported that *C. asiatica* inhibits the growth of human uterine carcinoma, human gastric carcinoma and murine melanoma cells *in vitro*. *C. asiatica* contains different glycosides like indocentelloside, theankuniside, brahmoside, asiaticoside, brahminoside, and isothankuniside. Asiaticoside is used for the treatment of tuberculosis and leprosy as well.

Having high therapeutic potential, it is very important in pharmaceutical industries which escort to its overexploitation resulting in the loss of the population of *C. asiatica* from their natural habitat (Nayar and Sastry, 1987). *C. asiatica*, thus recognized as threatened medicinal

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herb (Sharma and Kumar, 1998; Singh, 1989) is endemic to Western Ghats of South India (Nayar, 1996). So, this is why we need some alternative methods for conservation of this plant. Tissue culture technique is helpful for the mass propagation of plant and conservation of rare, threatened and endangered germplasm (Rao, 2004). Seasonal dependence and slow growth are the demerits of conventional propagation. Important features of *in vitro* propagation procedure are its enormous multiplicative capacity within a short time span; production of healthy and disease free plants; and its ability to generate propagules throughout the year.

Process of *in vitro* clonal propagation:

The success of micropropagation is influenced by different parameters. The critical points for the effective *in vitro* culture establishment are the efficient sterilization technique of explants. It is dependent on explants type, genotype, process of sterilization, different conditions like physical, chemical etc.

Choice of explants:

Explants choice is very important for production of true-to-type progenies. In *Centella* nodes or apical buds have been used as explants due to their genetic stability (Das *et al.,* 2008; Kaensaksiri, 2011; Karthikeyan *et al.,* 2009).

Sterilization:

Explants can be collected from those stock plants grown in a controlled environment, such as a greenhouse to get the better result. This process is better than the use of field-grown plants because it reduces microbial contamination (Webster and Jones, 1989; Yepes and Aldwinckle, 1994; Preece, 2001; Preece and Read, 2003). There are several methods for surface sterilization. Explants were thoroughly washed under running tap water and the leaves and roots were trimmed off from the plant. Shoot pieces were excised from the stolons and kept in tap water for 30 minutes. Then nodal explants were washed in mild detergent like 1% (v/w) Teepol, for 5 min with constant agitation. The explants were surface sterilized with aqueous mercuric chloride (0.1% $HgCl_2$) for 3 min. After this treatment the solution was drained off (Thangapandian *et al.,* 2012) followed by rinsing with double distilled water for 4-5 times. Kaensaksiri (2011) in his experiment**,** treated the 1.5 cm long shoot tips with a mixture of 2 g L^{-1} Funguran and 2 g L^{-1} Orthocite for 30 minutes and then soaked with 70 % EtOH for 60 seconds. Next, the shoot tips were sterilized with 3 % sodium hypochlorite solution containing 0.1 % Tween 80 for 10 minutes and rinsed thrice using autoclaved water. Cetrimide, bavistin and others could be use as sterilizing agent (Moghaddam, 2011)

Culture medium and culture conditions:

The most commonly used medium for micropropagation of *C. asiatica* was MS basal medium (Murashige and Skoog, 1962). Though application of other medium had also been

reported but MS medium was also efficient for *in vitro* culture. In general 3% (w/v) sucrose had been used as carbohydrate source. Carbohydrate in the medium serves as energy and carbon source. The pH was adjusted to 5.6-5.8 with 1N NaOH or 1N HCl. 0.8% (w/v) agar was used for solidification of medium and autoclaved at 121° C for 15-20 min. The cultures were incubated at 24±2° C under 16-hr photoperiod.

Shoot multiplication:

The success of *in vitro* propagation depends on the mode and rate of shoot multiplication. The of shoot bud multiplication is controlled by different factors, like genotype, composition of medium, different factors, PGR etc. Shoot multiplication is hormonally controlled mainly by cytokinin but sometimes in combination with auxin. Shoot multiplication of *Centella* is based on medium supplemented with cytokinin as the major PGR. The effects of different PGRs are genotype dependent. It is found that a mixture of cytokinin with auxin in 3:1 proportion resulted in better for shoot elongation. BAP in combination with IAA (4:1) showed good response in shoots elongation. In another report, within 30 days higher shoot multiplication was found in a media containing 1.5 mg l^{-1} BAP and 0.5 mg l^{-1} IAA (Thangapandian *et al.,* 2012). The highest percentage of multiple shoot bud induction was 76.67% on the medium augmented with 4.0 mg l^{-1} BAP+0.1 mg l^{-1} NAA followed by 70.00% on the medium consisting of 3.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA. Lowest percentage of multiple shoot bud induction (10.00%) was found on the medium containing BAP (7.0 mg l^{-1}) along with NAA (0.1 mg l^{-1}). The highest shoot development (10.2 ± 0.38 per explants) was obtained on the medium having 4.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA followed by 8.0±0.38 shoots per explant in the medium fortified with 3.0 mgl^{-1} BAP + 0.1 mgl^{-1} NAA. On the other hand, the minimum number of shoots was 1.1±0.20 per explants, obtained when cultured on the medium supplemented with BAP (1.0 mgl^{-1}) and NAA (0.1 mg l^{-1}). For shoots elongation, same medium was used. MS medium containing 4.0 mg l^{-1} BAP+0.1 mgl^{-1} NAA was found to be the best treatment for the highest multiple shoot induction as well as maximum number of shoots per explants (Das *et al.,* 2008). Similar results were also reported in other medicinal plants, such as *Celastrus paniculatus* (Martin *et al.,* 2006); *Heracleum candicans* (Wakhlu and Sharma, 1998); *Spilanthes mauritiana* (Bais *et al.,* 2002); *Coleus blumei* (Rani *et al.,* 2006). Kaensaksiri (2011) found that when explants (shoot tip) were cultured in liquid MS medium with thidiazuron (TDZ) (4.54 μM) for 15 days, 8.9 shoots developed per explant. Moghaddam (2011) proposed that the synergistic combination of use of BAP along with NAA in 2 mg/L and 0.1 mg/L concentrations respectively, resulted in sprouted shoots at optimum level 93 ± 0.667, shoot bud number 5.2 ± 0.079 per explants, length of shoot 4.1 ± 0.67 cm when cultured in Duchefa medium in comparison to MS medium. According to Tiwari *et al.* (2000) exogenous supply of BAP was required for bud breaking but the synergistic combination of BAP and NAA (22.2 μM and 2.68 μM concentrations respectively) was found to

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be optimum for shoot formation (91%) as well as number of shoot developed (4 to 5 shoots/ node). So BAP was most potent PGR in shoot multiplication of this plant. Subculturing enabled healthy shoot production. MS medium containing 6.7 μM BAP and 2.88 μM IAA was found most suitable for shoot elongation.

Rooting:

Successful root induction process before establishment in soil is a prerequisite for any propagation method. Auxin hormone was added exogenously to *in vitro* generated shoots promotes root formation. Tiwari *et al.* (2000) found that rooting was highest (90%) on MS medium (full strength) containing 2.46 µM IBA. According to Moghaddam (2011) rooting frequency, number of roots per shoot and mean root length occurred at optimum level (95.2 ± 0.81%, 7.5 ± 0.107 and 4.5 ± 0.133 cm respectively) when shoots that were cultured on MS medium (full strength) containing indole-3-butyric acid (IBA) 0.5 mg/L. According to Karthikeyan *et al.* (2009) 0-3mg/L NAA, IBA, IAA was also helpful for root induction. After isolation from culture tube, when well developed shoots were cultured on MS media having different concentrations of IBA for root induction. According to Das *et al.* (2008) the highest parentage of root induction was 90.00% on the MS medium with 1.0 mg l^{-1} IBA. After 40 days well rooted plantlets were obtained (73.33%) on the medium with 1.5 mgl^{-1} IBA. On the other hand, the lowest percentage of rooting was 10.00% on the medium augmented with 3.0 mg l^{-1} IBA. Highest number of roots per shoots was 10.6±0.93 from the medium augmented with 1.0 mgl^{-1} IBA followed by 8.2±0.96 roots per shoot on the medium with 1.5 mg l^{-1} IBA. On the contrary, the lowest number of roots per shoot was 0.8±0.06 in the medium fortified with 3.0 mg l^{-1} IBA. Use of IBA was the ideal treatment for root induction. Low concentration of IBA was more potent than that of higher concentration. Similar results were also reported in different medicinal plants, such as *Heracleum candicans, Plumbaga zeylanica, Cassia alata* and *Solanum trilobatum* (Wakhlu and Sharma 1998; Jawahar *et al.,* 2004; Chaplot *et al.,* 2006, Hasan *et al.,* 2008).

Acclimatization:

The transfer of plantlets from *in vitro* to *ex vitro* conditions is a very important step in the structural and physiological adaptation of plants; this is the beginning of the autotrophic life of plants. Micropropagated plants require their successful acclimatization and subsequent transfer to the field. Difficulties during acclimatization include rapid desiccation of plantlets and their susceptibility to bacterial and fungal diseases. There are several methods for acclimatization. Tiwari *et al.* (2000), in their experiment, removed plantlets from culture medium followed by washing the roots in tap water so that no agar is present in roots, plantlets were then transferred to a plastic cup that contain a mixture of sterilized garden soil or soilrite and were covered with

polythene bag. The potted plants were maintained inside a culture room at 24±2°C for 16 h/day illumination with cool-white fluorescent light (20 μmol $m^{-2}s^{-1}$). After 7 days, polythene bag was removed, then for another 2 weeks those plants were nursed in the culture room, followed by field transfer. According to Tiwari *et al.* (2000) the *in vitro* plants of *C. asiatica* were transferred into plastic cup containing only soilrite had 90% survival rate, after acclimatization, the plantlets were established in field successfully. Sivakumar *et al.* (2006) demonstrated that for acclimatization, well-developed rooted plants were transplanted to the tray filled with soil mixture, containing Canadian *Sphagnum* peat moss, perlite and vermiculite and were maintained in the growth chamber for two weeks. Then plantlets were transferred to the glasshouse and potted in red soil for another 2 weeks. Then plants were kept outside, and the survival rate was 95%. Kaensaksiri (2011) successfully acclimatized *in vitro* rooted plantlets at room temperature (30 ± 2 °C) for 1 week, and roots were washed with tap water, followed by transferred to plastic tray that contained a mixture of sand and rice shell ash (1:1), and grown in a tent where proper moisture content was maintained and the plantlets were kept for 21 days. Then, the plantlets were transferred into the greenhouse conditions for 7 days. The plants were then transferred to a plastic pot (6-inch) containing soil for 2 months and the survival rate was 86%. In another experiment, after removal from the culture vessels, the plantlets were washed with tap water and transferred to plastic pot containing a mixture of soil, sand, farmyard manure (1:1:1) (Das *et al.,* 2008). The plantlets were covered by polythene to maintain humid conditions. After proper acclimatization, the plantlets were then transplanted to the field, where 80% plants were survived. From all the studies it is clear that during acclimatization soil mixture played an important role.

Direct organogenesis:

Shoot regeneration was found when cultured on MS medium with NAA (0.1 mg dm^{-3}) and BAP (1.0 - 5.0 mg dm^{-3}) (Banerjee *et al.,* 1999). Direct shoot regeneration was highly variable, depending on the genotype and explant. For petiole explants, the mean maximum regeneration frequency (42 %) on medium supplemented with 2.0 mg dm^{-3} BAP and for petiole explants of another genotype, cultured on 0.1 mg dm^{-3} IBA, found maximum regeneration frequency (44 %) (Banerjee *et al.,* 1999). However, in other hand, for leaf explants, in one experiment, maximum regeneration frequency (30%) occurred with 3.0 mg dm^{-3} BAP and 0.1 mg dm^{-3} NAA, 25 % regeneration for leaf explants of occurred on medium containing 5.0 mg dm^{-3} BAP and 0.1 mg dm^{-3} NAA (Aziz *et al.,* 2007).

Indirect organogenesis:

In *C. asiatica* there are many reports which indicate rapid regeneration and multiplication through organogenesis. Callusing was observed at optimum level in MS + BAP (0.5 mg/l) + NAA (0.3 mg/l) using stem and leaf explants and the frequency of callus induction was 75% and

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83.33% respectively. For shoot induction from leaf callus, MS medium supplemented with BAP (0.5 mg/l) and MS medium supplemented with BAP (0.75 mg/l) when applied in the callus derived from stem explant were regarded as the most potent. Indole-3-butyric acid (IBA, 0.5 – 2.0 mg/l) showed the best response for root induction when used on MS media (full strength). Regeneration of multiple shoots was observed from callus derived from nodal explant when cultured on MS medium supplemented with Kn and IBA. Further when callus was subcultured on MS medium with IBA and different concentrations of Kn, optimum shoot growth was observed. Maximum callus production was observed on MS basal medium supplemented with IBA (3 mg/l) and Kn (3 mg/l) and maximum number of shoots were formed on MS medium with IBA (1 mg/l) and Kn (3 mg/l).

Somatic embryogenesis-

In *C. asiatica* there are many reports on somatic embryogenesis. By the process of somatic embryogenesis, ample numbers of plantlets to be produced within a short time. Somatic embryogenesis has been reported for many medicinal plants of Apiaceae, namely *Trachyspermum ammi* (Sehgal and Abbas, 1994), *Heracleum candicans* (Wakhlu and Sharma, 1998), *Eryngium foetidum* (Ignacimuthu *et al.*, 1999), *Daucus spp*. (Imani *et al.*, 2001), and *Cuminum cyminum* (Tawfik and Noga, 2002). Different stages of somatic embryogenesis are-globular, heart-shaped, torpedo, bipolar stage and from bipolar to plantlet formation**.** High-frequency of somatic embryo development and subsequently plantlet formation were achieved on callus derived from leaf, internode and stolon tip explants. PGRs significantly influenced process of somatic embryogenesis and subsequent plant regeneration. In one experiment by Martin (2004) callus developement was achieved on MS medium fortified with 4.52 mM 2,4-D or 5.37 mM α-naphthaleneacetic acid (NAA), both with 2.32 mM kinetin (Kn), were superior for somatic embryogenesis. Induction of embryo followed by maturation was much earlier when subcultured on medium fortified with NAA and Kn in comparison to that subcultured on Kn and 2,4-D. In another experiment, within 3 to 4 weeks of culture induction of somatic embryos occurred in the dark on medium supplemented with 2,4-dichlorophenoxy acetic acid (2,4-D). Leaves produced embryogenic calli at 2.26 and 4.52 mM 2,4-D, whereas stolon tips were responsive only in the 9.04 mM 2,4-D treatment. Embryogenic callus cultured on medium with NAA and Kn were transferred to liquid cultures of MS medium (half strength) supplemented with 2.69 mM NAA and 1.16 mM Kn, 204.3 somatic embryos developed/ 100 mg callus. While embryogenic callus cultured on 2,4-D and Kn and subsequently transferred to liquid cultures of MS medium (half strength) fortified with 0.45 mM 2,4-D and 1.16 mM Kn, 303.1 embryos developed/per 100 mg callus. 88% of the embryos underwent maturation and conversion to plantlets upon transfer to half-strength MS semisolid medium having 0.054 mM NAA with either

0.044 mM BAP or 0.046 mM Kn. Plantlets derived from embryoids established in field showed morphological similarity. The effectiveness of 2,4-D and Kn for somatic embryogenesis induction has also been well established among the other Apiacean members, for e.g., *Apium graveolens* (Williams and Collin, 1976), and *Cuminum cyminum* (Tawfik and Noga, 2002). The success of somatic embryogenesis depends on induction and maturation of embryos. In the study of Martin (2004) embryos from the callus developed on medium containing Kn and NAA exhibited early maturation and conversion as compared to the callus developed on Kn and 2,4-D. According to Joshee *et al.* (2007) withdrawal of 2,4-D resulted in maturation of somatic embryos and further development of plantlet from embryos. Peeters *et al.*, (1991) suggested that uptake as well as utilization of NAA is faster, so early maturation followed by conversion of embryoids may be due to the faster uptake as well as utilization of NAA. The delay in maturation and conversion of somatic embryos from 2,4-D induced callus may be due to its persistence in subsequent cultures. Zimmermann (1993) and Martin (2003) have also emphasized the persistence and inhibitory effect of 2,4-D in the maturation of embryos during consecutive cultures. Several factors control somatic embryogenesis such as Thidiazuron (TDZ) has cytokinin-like activity in different plants (Mok *et al.,* 1987; Visser *et al.,* 1992; Huetteman and Preece, 1993) and thus it is used in tissue culture. It is found that application of TDZ induce different responses such as callus induction, somatic embryo formation in many plants (Mok *et al.,* 1987; Visser *et al.,* 1992; Huetteman and Preece 1993; Zhang *et al.,* 2001; Ipekci and Gozukirmizi 2003). Somatic embryogenesis induced in presence of TDZ on the lower surfaces of cotton cotyledons was less genotype-dependent than when BA was used (Zhang *et al.,* 2001). Subsequent removal of TDZ from the callus induction media, showed embryo maturation and subsequent growth of the embryo that led to formation of plantlets on MS basal medium (Ipekci and Gozukirmizi, 2003). Friable callus developed on NAA, IAA, or 2,4-D alone and a combination of NAA or 2,4-D with BAP was inferior to NAA or 2,4-D with Kn-supplemented medium-derived callus in the efficacy of somatic embryogenesis. The callus developed on NAA or 2,4-D alone and in combination with BAP started to induce embryos 160 days after explant establishment and there was a reduction in frequency of somatic embryo development. Cream-colored friable callus developed on MS media supplemented with 4.52 mM 2,4-D and 2.32 mM Kn, upon transfer to suspension and solid cultures of MS medium (full strength) with reduced levels of 2,4-D (1.13 mM) and 2.32 mM Kn, exhibited proliferation of embryogenic callus only. Transfer of the embryogenic callus (90 d old) to liquid culture of MS medium (half strength) with reduced concentrations of 2,4-D (0.45 mM) along with Kn (1.16 mM) facilitated proliferation of embryogenic callus and development of embryos at 303.1 embryos/ 100 mg of callus. Embryonic callus cultured on MS agar medium (half strength) with 2,4-D and Kn at the same concentration under light was less competent for somatic embryo induction. Solid medium

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cultures incubated in the dark, formed fewer embryos than light-incubated calluses. White or ash-colored friable callus developed on MS medium with 5.37 mM NAA and 2.32 or 4.65 mM Kn was transferred to liquid culture with reduced level of growth regulators (2.69 Mm NAA and1.16 mM Kn) for somatic embryo induction. Somatic embryo induction was observed on subculture between 80 and 90 days after inoculation of explants and subsequent transfer induced development of somatic embryos in large number. During the period of subculture, nearly 204.3 embryos/ 100 mg callus were obtained in liquid of MS medium (half strength) with 2.69 mM NAA and 1.16 mM Kn. However, few embryos progressed to cotyledonary stage. Agar gelled medium with the same PGRs was less efficient in somatic embryos induction. Proliferation of the embryogenic callus was also observed and was more frequent on solid medium.

Low cost methods of micropropagation:

Low cost options were potent in lowering cost without changing the quality of plantlets (Raghu *et al.,* 2007). For establishing commercially feasible microprogation method, two combinations such as half strength MS and quarter strength MS medium were applied. For reduction of cost of agar, liquid medium, house hold cane sugar as carbon source, jam bottles and, tap water were used as it is less expensive (Raghu *et al.,* 2007).

Applications:

Synthetic seed:

Synthetic seed (= synseed) technology, based on the use of different micropropagules like somatic embryos, axillary shoot buds, apical shoot tips, embryogenic masses and protocorms, provides methods for production of seed analogues. It can be an alternative to traditional micropropagation for production of cloned plantlets in the future although it still has limitations for wider commercial uses. Artificially encapsulated micropropagules are able to convert into plantlets under controlled conditions and can be used as a seed after proper storage. For embryo encapsulation, 1%, 2%, and 3% sodium alginate (w/v) were dissolved in basal MS medium with sucrose (3%). Embryoids of 1.0 to 2.0 mm long heart shaped stage and bipolar stage were subcultured on MS basal medium without any PGRs, were used for encapsulation of embryo. Embryoids were transferred to alginate mixture (0.15 to 0.20 mL). After encapsulation with alginate, calcium chloride solution (50 mM and 80 mM) was added drop wise. Every drop with one embryo was nursed in calcium chloride solution for different times like 10 min, 30 min or 60 min. After incubation period, calcium chloride solution was decanted and followed by recovery of beads. Beads were washed thrice with MS basal solution. Somatic embryos encapsulated with sodium alginate (2%) prepared in MS salt solution and submerged 30 to 60 min in 80 mM calcium chloride for hardening, produced seeds with a coat firm enough for handling and

allowing synseeds to develop into plantlets. It was observed that 30 minutes exposure in calcium chloride solution (80 mM) was good enough for production of firm synthetic seeds. The germination was influenced by sodium alginate concentration, same type of observation was found in many other plants also (Rao and Singh, 1991; Castillo *et al.*, 1998; Malabadi and van Staden, 2005). Synseeds with sodium alginate coating (2%) showed the best results (60% survival) for germination than the other treatments. Storage at low temperature was important for conservation purpose. Further study using cryoprotectants and different types of soluble and insoluble sugars in the encapsulation matrix may be helpful for longer storage and better germination and survival. In another report, encapsulation with sodium alginate (3%) in *Paulownia elongata*, showed 73.7% survival rate and 53.3% germination frequency. These results are promising and suggest that synseed technology offers great potential both in micropropagation and in germplasm conservation, although further research is needed to increase the rate of conversion and to develop steps towards automatization.

Production of bioactive compounds:

In the liquid-culture the production of asiaticoside was higher amount [494.62 mg g^{-1} (d.m.)] than that of callus and leaf [190.48 and 125.0 mg g^{-1} (d.m.)] tissues. So, using suspension culture, large amount of callus was obtained within a short time and the alkaloid content increased too. Thus, by suspension culture it is possible to achieve higher growth and production of compound (Nath and Buragohain, 2005).

Biomass (30 g) was collected from shake culture and then was transferred into a bioreactor (5 L). Proper agitation was maintained at the rate of 120 rotations per min for 30 days. Aeration (2 L/min) was achieved that helps better production. The culture was maintained at 25°C temperature. At agitation speeds of 170- 200 r/ min reached asiaticoside content was low, while asiaticoside content reached highest value of 59.43 mg/g dry weight at 150 r/ min.

Genetic transformation through hairy root cultures:

For overcoming conventional, sexual hybridization systems, the use of genetic transformation is essential in the genetic improvement. Genetic engineering plays crucial role in the efficient transfer of horticulturally important features like resistance to bacteria, insects, fungi and herbicides, rooting ability, or dwarfism of rootstocks, environmental stress resistance or modified metabolism, from various genera. Successful application of genetic transformation is based on the development and existence of tissue culture techniques. Hairy root culture is very good approach for secondary metabolites production. It is reported that by hairy root cultures using *Agrobacterium rhizogenes* strain R1000 asiaticoside and madecassoside production were increased. It is also seen that hairy roots were formed from a tissue junction between the leaf and petiole (14.1%). Co-cultivation for 7 days with *A. rhizogenes* resulted 36.1% production of hairy roots. When elicited with MJ treatment asiaticoside production was enhanced (Kim *et al.*, 2007).

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Asiaticoside biosynthesis shown to be tissue specific, produced mainly in the leaf of *C. asiatica* (Kim *et al.*, 2004). In addition to these, the CabAS transcripts (putative b-amyrin synthase) gene, associated with asiaticoside biosynthesis, was detected in leaf tissues only (Kim *et al.*, 2005). Nath and Buragohin (2005) reported the interesting fact that sizeable quantities of asiaticosides are biosynthesized in suspension-cultured cells and undifferentiated calli of *C. asaitica*. Application of elicitor to hairy root cultures of *C. asiatica* resulted into better asiaticoside production. An observation similar to this result has shown that in *in vitro*-cultured roots of whole plants treated with MJ, asiaticoside contents were about five times than that of the controls. On the basis of these results, it is found that there is a positive correlation between high levels of CabAS mRNA and asiaticoside production.

Conclusion:

In vitro regeneration of *Centella asiatica* indicates that it is feasible for rapid propagation, faster introduction of new cultivars with desirable traits and for rapid multiplication of disease-free, healthy propagation material. The studies carried out during the last several years on different stages involved in micropropagation of *C. asiatica* has led to considerable improvement of protocols and methods. Use of liquid medium shows cost efficiency in micropropagation using bioreactors. Results reviewed on propagation of *C. asiatica* in bioreactors indicate that it can be the most promising way for production at large-scale in the future. However, some new challenges are faced day by day by the tissue culture industry that includes cost efficiency, automation, control and optimization of the microenvironment etc. *In vitro* regeneration of *C. asiatica* via somatic embryogenesis, direct regeneration and indirect regeneration offers a great potential for rapid propagation and improvement.

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Impact of Pesticide Application on Insect Pollinators: A Review

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Abstract:

The apiculture industry is the agro-based industry completely relies on the honeybee. The industry gives many apiary products in the form of honey, beeswax, pollen, royal jelly, propolis and bee venom which has great demand in the food, cosmetic, medical and pharmaceutical industry. Despite this, honeybees are the efficient pollinators which provide crop pollination service overall the world. It increases crop productivity in qualitatively and quantitatively. But day by day, the honeybee colony disappeared due to several reasons; the immense use of pesticide in agriculture field is one of them. Pesticides show negative impact on the foraging efficiency of honeybees due to which the colony becomes weak and shows adverse impact on their pollination as well as apiary production. Hence the conservation point of view, it is necessary to monitor and minimize the pesticide application by maintain healthy coordination with the farmers.

Keywords: Honey bee, pollinators, impact of pesticide, conservation.

Introduction:

Pollination is an important natural service provided by the wind, water and animals, resulting in sustainability and continuity of the ecosystem. Among these, more than 75% of the agriculture crop species of the world rely on biotic pollinators for fruit and seed set. The biotic pollinators includes insects and many vertebrates including birds and mammals which collecting the nectar by visiting many flowers and accidently transfer pollen from male to female flowers.

Pollinators contribute 35% to global food volume and play a key role in supplying vital nutrients for human subsistence directly or indirectly (Kearns *et al.,* 1998; Klein *et al.,* 2007). The pollinated products include many fruits, nuts, vegetables, fiber crops and oil seeds. They benefit society by increasing food security and improving livelihoods and by the role they play in conserving biological diversity in agricultural, forest and natural ecosystems. Among the biotic pollinators, insect pollinators are the major source of pollination which includes hymenopterans

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(honey bees, solitary bees, bumblebees, wasps, and ants), dipteran flies (bee flies, hoverflies and mosquitos), lepidopterans (butterflies and moths) and some flower beetles. Among these, honey bees are only insects which provide food for human beings (Baskar *et al.*, 2017).

Honeybees are the social insect lives in a colony which shows healthy and effective impact on the agricultural and forest production. Due to high population, perfect body pattern and safe landing on flowers without any damage to the floral parts, they pollinate thousands of flowers in a small period. Day by day apiculture industry flourished with not only increasing crop productivity but also increasing the apiary production in the form of honey, bees wax, pollen, royal jelly, bee venom and propolis. But another side, more than 40% of honey bees have been disappeared during last 25 yrs in India (Gallai *et al.,* 2009). It may cause scarcity of food in the form of fruits, vegetables and agriculture crop which are pollinated by the bees. A decline in honeybee colonies in India is a severe problem which may be due to increased use of pesticides, deforestation, industrialization, urbanization, monoculture, infestation by diseases and predators, mobile tower radiation effects and lack of awareness.

Pesticides is one of the major threat for honeybees where the colony becomes weak and shows adverse impact on their pollination as well as apiary production.Hence it is necessary to monitor and maintain the application of pesticides due to which it is convenient to make the apiary industry sustainable.

Application of pesticides:

Pesticides are the chemical substances used to eliminate unwanted pests from the various sectors where the population of insects increases above the threshold level. In other words, it is the chemical or their mixture applied for preventing, destroying, repelling or mitigating any type of pest. The term pesticide is applies to insecticides, herbicides, fungicides, and various other substances used to control pests. Pesticides may include plant regulators, defoliants and desiccants (Johnson *et al*. 2010; Simon-Delso *et al*. 2014). In India, from the past few decades the farmers use various external efforts, including fertilizers and pesticides for the reduction of crop infection and also to increase the crop productivity as per the nation demand.

The massive use of pesticides in the agriculture ecosystem results in declining the population of honeybee and other pollinators throughout the world (Krupke *et al*. 2012; Lebuhn *et al*. 2013; Chakrabarti *et al*. 2015).In the United States, due to immense use of DDT in agriculture,the colonies of European honey bee, *Apismellifera*were reduced from 6 million to less than 3 million (Ellis, 2012). It was also reported that around 14% honeybee colonies decline in

Scandinavia while 25% decline in central Europe (Potts *et al.,* 2010; Williamson *et al*. 2014). Many pesticides that have been banned in the developed countries, such as DDT, BHC, Mancozeb account for more than 46,000 tones and these are still being used in India. Contaminated pollen, nectar and water brought by bees to the hive from pesticides treated areas cause extensive damage to the brood and affect the growth of colonies.

Types of pesticides:

Among the pesticides, the insecticides are directly applied as sprays or dusts on the agriculture crop, while herbicides andfungicides are applied directly on the soil before the planting of crops. The insecticides applied in the agriculture field are categorized on the basis of mode of entry as contact poison, systemic poison, stomach poison and fumigants. The activities of the insects may facilitate the entry of toxicants into their vital organs, bring about various physical or physiological disorders and even cause death.

The sprayer and dusters are the appliances utilized to release fine particles of these pesticides in the agriculture field. But with the help of wind carried the tiny droplets and fine dust particles, can spread and fall directly on the bees located across the treated fields or away from the crop area. Such few particles of concentrated contact or stomach poisons may be effective for the death of a bee (Craig *et al.,* 1998; Marinelli *et al.,* 2004).

Contact poison kills the pest when come in contact and then absorbed by the body surface of the pest.It is applied as dusts or sprays.It kill the insects by Clogging spiracles and respiratory system or acting as nerve or general tissue poisons, after entering into blood through cuticle. Certain organochlorines (DDT, BHC, Aldrin), Organophosphates (Malathion, Parathion), Carbamates (Carbary, Dimetan) highly applied in the agriculture field.

Stomach poison is applied against the insect pests with chewing mouthparts which feeds on plant foliage.During feeding, the poison enters into the stomach of pest along with food material which then absorbed and circulated in the body and cause severe effect with death. It is applied as sprays, dusts, or baits. Lead arsenate, Calcium cyanide, Paris green, Organochlorines, organophosphates, carbamates is used as stomach poison.

Systemic poison is the chemical which penetrate and translocated to various plant parts and then enter the body of the insects which feed on them. It is applied on seeds, roots, stems or leaves of the crop plants. It is effective against insect pest with piercing mouth parts and suck cell sap by proboscis. Demeton, Phosphorothioate, Thimate or Phorate (organophosphate) are popular pesticides used as systemic poisons against many agricultural pest.

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Such pesticides when introduced into the soil by using many drilling appliances, particles of insecticides may detach from the treated seed and air becomes polluted. These poisonous particles can cause death of the bees. In addition to this, insecticides transfer in all the parts of the plant by the vascular tissues including the flowers, pollen and nectar. The pollinators especially honeybees when visited such treated crop plants for the collection of pollen and nectar from the flowers, the residues of insecticides transfer into the body which affected severely and cause death of the honeybee (Bonmatin *et al.*, 2015).

The effect of pesticides on bees:

Pesticides treated field may produce several effects i.e. indirect or direct. In indirect effect, the foraging activity of honeybees is reduced due to repellent effect of pesticides which shows sub-lethal toxicity. It may influence the bee behavior pattern as flight velocity, foraging speed, wing beat frequency etc. (Brandes, 1984).

Drifts of pesticide droplets in the comb may accumulate in stored pollen and honey and may cause stress or reduce the longevity of the bees. Such contaminated food may cause bees to cease feeding or there may be reduced consumption and collection of nectar (Fikadu, 2020). Another is the direct effect when bees directly come in contact with pesticide during foraging where the bees immediately dies and does not return to the hive. In this case the queen, brood and nurse bees are not contaminated and the colony survives (Maini *et al.*, 2010). As the bees comes in contact with pesticides and transports it back to the colony, the contaminated pollen or nectar or pesticide particles attaches to its body affect the other members of the colony and shows severe loss of the colony. When poisoning is severe, thousands of dead bees will accumulate in front of a hives (Sanchez-Bayo and Goka, 2016).

Poisoning cause the bees to become agitated and aggressive. They become hyperactive around their colony. Some other symptoms in bees are paralysis, abnormal jerky movement or crawling around the hive which has adverse effect on the foraging ability of bees. In such cases, bees quickly lose the ability to fly and die within 2-3 days. It also shows lack of coordination in the body organs such as legs, wings and abdominal movement, irregular egg laying capacity by queen, brood emitting foul smell, colony collapse disorder, absconding etc. (Mishra and Sharma, 1988). Overall the effect of pesticide is directly influence on the loss of colony as well as the apiary production. According to Gallai *et al*. (2009), more than 40% of honey bees have been disappeared during last 25 yrs in India. This is due to immense use of pesticide in the agriculture

field. A decline in pollinating insects in India is resulting in reduced fruit, agriculture crops and vegetable yields.

Initiative should take:

a) Time of pesticides application

The honey bees visited to the flowers (forage) takes place during daylight hours. As the sun begins to set, they return to their hives with fully loaded with pollen and nectar also. Little foraging takes place early in the morning or late in the evening. Thus, the application of pesticides during late evening or very early morning provides relative safety andcan significantly reduce honey bee mortality (Hameed and Singh, 2002). In case of sunflower and onion, the bee foraging activity is greatly reduced in the afternoon and hence application of pesticides can be treated in the evening hours.

b) Selection of pesticide formulation

During application of pesticides in the agriculture field, it is necessary to apply proper formulation of pesticide. The appropriate formulation is effective to minimize the mortality of honey bee.

Dusts and wettable powders are highly hazardous to bees because of their tendency to drift to a considerable distance and particles remain adhered to plant surface for long. As the bee come back to the hive with small particles that adhere to the hairs of the body and stored along with the pollen in the comb cells. During feeding to the queen and the brood, contaminated food can cause an entire colony to collapse.

The emulsifiable concentrates and the granular insecticides are relatively safer for the bees. They dry quickly and do not leave a powdery residue unlike the dusts and wettable powders. The insecticides with fumigant effect may also be hazardous to bees. The granular insecticides with systemic action may contaminate nectar and may result in losses to bees, foraging upon them (Free and Williams, 1979; Guenther and Dallman, 1968).

c) Pesticides selection

Selection of pesticides with less toxicity and rapid degradation has little effect on the honeybees. Some of the pesticides now days are available in the market as Endosulfan, phosalone, fluvalinate, lindane and so on. Application of these pesticides in the agriculture fields, it will take few hours to few days for complete degradation.

d) Method of application

The pesticides usually applied in the field by aerial or ground methods which may shows variable effect on honeybees and many beneficial insects. Aerial applications of pesticides are

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more hazardous to bees than ground applications. Most of bees get very short time to escape from the pesticide drift during foraging and may kills in the treated agriculture fields. Fine sprays are safer than coarse sprays.

e) Monitoring weather condition

Warm and sunny weather is considerably effective for foraging activity than dull weather. As prolonged dull weathersignificantly reduce the foraging activity of bees and safer for the application of pesticides in the agriculture field.

f) Distance of colonies

The distance of bee colonies from the pesticide treated field is also effective to reduce the risk of pesticide poisoning. To maintain the population of the colonies, put the colonies at least 3 km away from the treated field by pesticides.

g) Collaborative approach

Monitoring the colonies by beekeeper also maintain the healthy environment in the colonies. Beekeeper may transfer the colonies where pesticides are not used. As moving are impossible, covering colonies with a well-ventilated screen to restrict honey bee flight during peak foraging hours. Providing water and pollen inside the screen will allow the bees. If the colony is susceptible to the pesticides thencontinue provide the colonies with sugar syrup, pollen, and water.

As the colonies became weak then combine the weak colonies. It should be need of healthy cooperation between the farmers and beekeepers due to which reduce the loss of bees and the crops to be pollinated can be saved. The entomologist needs to develop integrated pollinator and pest management technologies which could reduce pesticide damage to pollinating insects. The problem of industrial toxicity and hazardous gaseous discharge, pollutants need to be solved by the scientists and experts. Besides these statutory laws restricting indiscriminate use of pesticides, precautions like notification of spray operation, movement of colonies will show desired effects.

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A REVIEW OF MIDGE FLIES CECIDOMYIIDAE (=ITONIDIDAE): DIPTERA

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Introduction:

Family Cecidomyiidae (=Itonididae) is one of the largest but youngest family of Nematocerous Diptera, having worldwide distribution. Insects commonly known as gall midges are included in this family. They are best known as plant gall makers. Many of them produce characteristics galls i.e. deformities on various plant parts on both dicotyledons and monocotyledons plants (caused by gall – midge of subfamily Cecidomyiinae), however members of subfamilies Lestremiinae and Porricondylinae do not produce such galls. Gall midges have phytophagus, saprophagus, xylophagus and predaceous feeding habits. A few of them are serous pests of economically important crops such as jowar, rice, bajra, wheat; fruit trees such as mango, citrus, papaya; oilseed crop such as linseed, safflower and several grass species. Some of them are predators of Aphids (Aphidomyia) , Scale insects, and mites also. Thus certain gall-midge species have role in biological control of a few serous insect pests. Larvae of gall midge show peculiarity of feeding habits as fungivory, herbivory, and predators of many arthropods.

Family Cecidomyiidae originated in ancient time and fossils were reported to be from old era of Jurassic period. Gagne (2004) reported 5451 species of from world. Majority of the gall-midge species are associated with flowering plants. Numerous species of gall midge are yet to be discovered from the world.

Classification of midge flies:

These minute flies belong to Order Diptera of class Insecta. They belong to family Cecidomyiidae. This classification followed all over the world. Gagne (2004) for the classification of Lestremiinae and Poricondylinae followed Panelius (1965) and Jaschhof (1998). He for the Cecidomyiinae use supertribe, tribes and subtribes.

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Subfamily: Catotrichinae

Subfamily: Lestremiinae

Subfamily: Porricondylinae

Subfamily: Cecidomyiinae

Subfamily Cecidomyiinae:

It is the largest subfamily of gall midge having 4281 known species. Gagne (1994) divided the subfamily into 4 supertribes viz.Brachineuridae, Cecidomyiidi, Lasiopteridi and Stomatosematidi.

The major contribution on Cecidomyiidae was started in 1926 when M. S. Mani began work on this group. Trained in taxonomic work with Dr. H.S. Pruthi and inspired by workers like Kieffer, Felt and Barnes, he made significant contributions to taxonomic study of the galls and the gall formers especially Cecidomiids. He worked on this group for nearly fifty years. Mani (1934, 1935, 1936, 1937, 1938, 1942, 1943, 1945, 1946, 1947, 1948, 1952, 1953, 1954, 1963, 1966, 1973, 1974), erected seven new genera and described sixty one new gall midge species collected from different host plants. Mani (1946) published the key to genera of midges from the oriental region to replace the earlier keys prepared by Kieffer (1913) and Felt (1925) along with the text figures. He erected the genus *Amradiplosis* in 1947 and enlisted sixteen midges infesting mango, out of which thirteen were identified. He explored the midge fauna of south India, high altitudes of Himalaya and also described Pusa collections. He reported many economically important midges on different cultivated plants.

Sharma (2009) worked on the gall midge fauna of Marathwada. He published a paper on midge galls of Andaman Islands. This paper deals with exomorphic descriptions of 35 midge galls with 24 plates. These galls are collected from 25 different localities of Andaman Islands. Of these 35 galls, 27 are new to the India. Key and gall index are also provided in the paper. This is the first report of galls from Andaman Islands. He has also reported many new gall midges from Pune, western-ghat, Raigad and Melghat reservoir (Maharashtra); Nilgiri reservoir (Karnataka) and Hissar (Haryana). He published a check list of plant galls of India (2003) and also check list of gall midges (2003) that occur in Maharashtra (2003). He has reported the occurrence of 379 species from 138 genera from India (2006).

Deshpande *et al.* (2002, 2005) reported two new genera *Garugadiplosis* and *Kitella* from Nanded (Maharashtra). They also reported revision of genus *Anaretella* Enderilen. A new midge

gall occurring on *Garuga pinnata* Roxb was reported from Bhokar forest of Nanded (Maharashtra), which is the first midge gall on the host tree.

A total of 394 species belonging to 125 genera under 3 subfamilies are so far enlisted in India (Sharma, 2009).

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NUTRITIONAL ENHANCEMENT IN SEA FOOD BY VALUE ADDITION

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Introduction:

The value addition in fish and fish products or in general any seafood has become a viable means in fulfilling the needs and demands for a good quality seafood all around the globe. The value addition in seafood further amplifies the need and importance of the seafood industries and consequently employment opportunities. The value addition is basically aims to enhance the nutritional value before it reaches out to the consumers. It starts from the time when fishes are caught and ends when the final value added product reaches the consumers.

Without proper cold storage arrangements, due to the limited shelf life, the chances of spoilage of fish are very high. This forces the fishermen to sell the raw fish without value addition before spoilage. Hence value addition becomes a viable means which can lead to a two end benefit both to the fishermen who can get competitive rates comparable to the local market and the consumer as well who will be consuming a more nutritious product. Therefore fish value will not be compromised at the expense of profit if the fishermen are provided with proper cold storage equipments.

There are plenty of benefits associated with value addition and fish processing, primary ones being the better and safer consumable product, improving the edibility by quality improvement, increasing the shelf life and also improving the margin of profit to the seller and the processor. The optimization between safety standards in terms of edibility and the completely new product synthesis that can produce pretty decent outcomes as long as compromise on the quality is not made. The value addition is not limited to mere processing and creating new packaging methods. It is a broad term which is inclusive of sustainability and ecological handling which is essential in the development of new markets and their expansion. The need for health driven products which can easily handle the seafood products has become the area of attention these days. With the evolving technologies and the growth in aquaculture domain have shown new opportunities for a large variety of products. Value added seafood products play an

essential role in fulfilling the demand of consumers by providing safe, high standard quality product.

The value addition is one of the prime topics of discussion in the processing industry, mainly in the export based processing industry due the viability of important foreign exchange. The value addition can be done in the fish and its products depending upon the market requirements. The wide span of variety includes from the very live fish to the consumer preferred product. In the fish processing industry the value addition is the most essential conventional approach to improve the profit generation with the cut throat competition and highly shooting up costs.

The large amount of fish and shellfish are normally discarded into the sea because of the economic factors involved in preserving them and bringing them up in the ashore. Shrimps by catch are well known example. The estimated amount of discard of the by-catches in nearly around 20-40 million tons/ year with the average of around 28 million tons/year. Factors hindering the bringing of the shrimp by-catch to the ashore is the low market value associated with the material, the composition of species and its size, lack of availability of proper refrigeration arrangements and the decrement in the efficiency of shrimping. In India, the gap between the maximum achievable potential and the current exploitation is mostly met through the deep fishing in the sea. The evolving interest in the deep fishing in EEZ (Exclusive economic zone), plenty of fishes deep under the sea are yet believed to land which may not immediately go with the consumer liking due to unawareness about the shape, color, size, odour and flavor of the newly brought varieties. The large development of the minced fishing technology over the past few decades can possibly be the driving factor in the exploitation of the deep sea fish variety and also by-catches. Fish consumption can be improved by utilizing the existing catch effectively. Because of the dearth of infrastructural availability in the developing countries, the quality of fish degrades, which limits them to their full potential use and it merely remains as an aquaculture feed. Improving the infrastructural set up can landing qualities can be transformed for the direct consumption to the customers. Such up gradation of the species can be achieved by employing more improved handling techniques and processing methods as well as synthesizing variety of products on other by value addition which can lead to more effective utilization of uniform distribution of species with same resources.

Value added products can broadly be classified as

1. Mince products
2. Breaded and coated products
3. Surimi based products

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In the minced products, is essentially the fish that is free from scales, bones and skin. The fish is gutted, washed prior to the mince preparation. Fish mince finds variety of application in several foods. The main advantage involved is that the identity of original fish remains unknown or hidden to the consumers, and they might accept the minced products even if the original fish may be not preferred to them as whole. A batter is the liquid mixture of water, starch and flour and the food products are dipped into it before cooking. Breading is basically bread fragments; however other coatings like potato chips and grains are also used widely. Surimi is basically the Japanese originated term which refers to the deboned fish mince from the white flesh fish that is washed, refined and also mixed with the cryptoprotectants for improving the shelf life due to the much higher gel strength; it is used as an intermediate in the processing of various value added products.

Fish Surimi

There is a very large demand of the ready to eat form of the seafood based products. A variety of such products have already reached the markets. The involvement of woman into this business and obtaining employment is one of the key factors is market expansion of these variety of products. The marketing of the products obtained from value addition is totally different the conventional seafood business. It is more complex, costly, and sensitive to risks. Market and customer surveys, quality packaging and branding and marketing ultimately decide the success of the product in the market. Most channels which are currently employed are not well suited for an effective trading of value addition products. An effective channel can possibly be the market chains which can direct purchase from the supply source. The success of product evolved from value addition is essentially the general appearance, quality of packaging done. The retail labels must be clearly depictive of the contents in the product, which can please the customer to buy. An effective communication can convince the consumer to try the new product launched. Packaging may change with the products; packaging with the modern technology may keep the product at par with the conventional products.

Value addition in fish:

Value process generally includes:

- Processing which involves chopping the fish by fishermen
- Proper packaging of fish
- Preserving the fish in cold storage
- Smoking, drying by solar power
- Acid addition to prevent microbial growth

Challenges faced by small fishermen:

- They often encounter problem of cold storage facility particularly at fishing site
- Connectivity through roads to the ashore from the fishing site
- Improper market for fresh fish in local area
- Financial issues make it hard to purchase and employ modern equipments
- Lack of knowledge and awareness about the importance of value addition in fishes
- Lack of capability to foresee the demand and consumer preference and expectation with respect to the quality and consistency

Consumer preference:

There is always the expectation from the consumers toward the retailers that the product that is being given is of utmost good quality as well as safe to consume and also derivate of the fisheries that are sustainable. From the retailer end in order to be able to provide such guarantee, they must receive along with the seafood, the certification of the completeness of the product in terms nutritional value, health benefits, the proper food label which can clearly depict the contents in the product, label which gives the authenticity of its origin from sustainable fisheries. So the large scale retails demand the certification according to their own standards and schemes in terms of both food safety and sustainability. As the things will unfold in future it is expected that aquaculture will certainly meet the market demand and products will be of very high quality. The perception about any product to the consumer largely depends on the beliefs, origin and processing technologies that are being used. This information is very sensitive and prone to misapprehension, hence must be dealt and communicated in an effective manner.

There have been significant changes in the preferences of the consumer in past few decades. The demand for value added products in domestic markets have been in proportion with the growth of middle income group particularly in metro city. It is therefore necessary for the

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industry to deal accordingly with the changed trends. Creating such market in domestic arena could possibly be result in stagnant varieties available; it will be sensitive to fluctuations in the demand supply cycle and it will lead to total dependence on export market.

It is expected in upcoming days, for the domestic demand a part of seafood catch disposed fresh, may be utilized for processed products in different forms. With the increasing life standards, the people in general have little or no time to prepare food in a conventional way. Brazil is one of the examples for this. People in South America prefer to enjoy the food in a traditional way as a passion or hobby. Whereas people in North America, they are not so bound toward the tradition when it comes to food. As the home made meal replacement grows, so the quality standards associated with it. The type of raw material that is used, companies associated with production of these. The general increase in demand prompts the need of food safety and improvement of quality standards. There are many factors which affect behavioral relationship but the considerations like the convenience, the added value, price, readily availability certainly influence consumer in actual purchase or buying scenario. Taste, dislike, nutritional value and freshness of the product are suggested to be important factors in altering customer opinion and preferences towards the seafood. Also the consumer need depend majorly on their age groups.

Marketing of value added products:

Marketing of value added products is completely different from the traditional seafood trade. It is dynamic, sensitive, complex and very expensive. Market surveys, packaging and advertising are a few of the very important areas, which ultimately determine the successful movement of a new product. Most of the market channels currently used is not suitable to trade value a5ded products. A new appropriate channel would be the super market chains which want to procure directly from the source of supply. Appearance, packaging and display are all important factors leading to successful marketing of any new value added product. The retail pack must be clean, crisp and clear and make the contents appear attractive to the consumer. The consumer must be given confidence to experiment with a new product launched in the market. Packaging requirements change with product form, target group, market area, species used and so on. The latest packaging must also keep abreast with the latest technology.

Value addition of process discards:

Invariably, the term ‘waste management’ in fisheries sector is gradually changing to ‘value addition of process discards’. A considerable quantity of fish waste is being generated

from processing operations, which may go as high as 70% of raw material, and is even more costly to dispose. Considering the proteinaceous nature and abundance of biomolecules of medicinal and therapeutic value, fish processing waste is classified as 'a certified or prescribed waste'. Process discards, which is primarily composed of head, scale, skin, bone, viscera, fins, eyes, and gills is a major reservoir of high value by-products such as protein, fish oil, PUFA, collagen, gelatin, bioactive peptides, chitin, chitosan, glucosamine, calcium, pigments, hydroxyapatite etc. Additionally, the bulk reduction technologies may be adopted for the conversion of these discards to protein and mineral rich farm inputs such as manure, fertilizer, foliar spray, aquaculture feeds, poultry and pet feed etc. through less capital intensive methodologies. On the foremost, a successful waste management programme depends on the strengthening the baseline data (waste generation, local facilities, current disposal plan, major stakeholders etc).

Lack of clear legal classification of secondary products in the international market, lack of unified protocols for quality assurance (such as HACCP) and vitally, lack of awareness about value generation opportunities from process discards are certain key elements restraining the advancement of this segment. Development of efficient means of networking and establishing inter-industrial linkages between potential stakeholders (timely follow-up and review of the efforts undertaken is a must), generation of mobile pilot technological platforms for testing and demonstrating different technologies, encouraging more public-private- organizational partnership (incubation centers for pilot production), introducing public policies and legislations against waste dumping, framing policies for better use of fishery wastes (such as coupling of licensing of markets and processing facilities with waste conversion measures taken at the source of generation) are certain indicative strategies for overcoming the current scenario.

Future perspectives:

The current status of captive farming in Asia, especially in cages and pens, and the developments witnessed globally suggest that the farming of high value fishes such as seabass, mullet, pearl spot, silver pompano grouper, cobra and red snapper has a bright future. They have several desirable traits, most importantly a rapid growth rate under tropical climate and good flesh quality. Similarly, many of the molluscan candidates, in particular, mussels and oysters have captured Wide popularity in recent years. As the top leading countries in aquaculture, the future production from China and India is likely to be a major factor in popularization of these species for commercial processing operations. Countries such as Vietnam, Thailand and Indonesia have gone far ahead in coastal farming, while India is yet to make a mark, even though

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we are having a vast coastal line and all the favourable climatic advantages of a tropical country. This increasingly demands viable processing, product diversification, value addition and live transportation options for channelizing the harvested resources to domestic and international markets, which is expected to make significant increase in the country's foreign currency earning.

The immediate benefit expected through brackish water/saline water farming promotion is minimization of the dependency on marine capture fisheries for raw material supply, which is already in a highly stagnated and more or less in a depleted status. A long term benefit envisioned is to present a viable option for the minimization of use of hazardous preservatives, as practiced nowadays for extending the holding time of raw material with vendors. In the case of capture fisheries, which relay on multiday fishing operations, the material is stored for more than a week before it reaches the landing centers. Unlike this, in culture fisheries the processor or the vendor is on a beneficial Side that the harvest operations can be scheduled according to the market demand so as to get the material on-board at 'zero storage time'. This delivers them more retainable period, and in turn can refrain from the use of hazardous preservatives. ICAR-CIFT has initiated a three year project on developing handling and processing protocols for emerging farmed fishery resources with emphasis on coastal enclosure systems. The project covers the various aspects on species specific on-farm handling protocols for brackish water and mariculture species, novel value addition options and live transportation protocols.

Undoubtedly in long run, technology up-gradation will continue to remain as the key element in value addition domain, supported by minimal processing options for maximum nutritional retention, innovative ideas of packaging, intelligent systems for quality monitoring etc. High Pressure Processing, even though not increasingly invaded in seafood sector currently, is expected to gain fast pace in coming years as a non-thermal mean to extend shelf life. Out of box thinking and product development addressing niche markets is an important strategy for improving farm income. Transferring such technologies with a viable business model has the potential to start new industry and generate additional income to the farmers. Inevitably, a solid effort for encouraging value addition in seafood sector emphasizes the primary requirement of appropriate mechanization for reducing drudgeries, up gradation of capacity and efficiency for cold chain system, as well as development of intelligent and smart transportation techniques for increased shelf life. Moreover, parallel developments should be reflected in Government policies and investor friendly incentives, in order to make the industry globally competitive.

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RELATIVE STUDY ON ANTIBACTERIAL ACTIVITY OF DIFFERENT FUNGUS SYNTHESIZED SILVER NANOPARTICLES AGAINST BACTERIA

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Abstract:

In this study we investigated the antibacterial effects of three fungi (*A. flavus*, *A. fumigatus* and *A. niger*) synthesized silver nanoparticles (AgNPs) against *Bacillus subtilis* and *Escherichia coli*. Agar well diffusion technique was used for investigation. The characterization of fungus synthesized silver nanoparticles down by UV-spectroscopy found absorption peak at 450, 400 and 450 nm respectively. TEM micrograph showed polydisperse spherical and some are ellipsoidal nanoparticles ranging from 1-50 nm in size. The present study indicates three species *Aspergillus* genus synthesized AgNPs has potent antibacterial activity against gram positive and gram negative bacteria comparison with antibiotics. It was observed that silver nanoparticles of three species of *Aspergillus* genus showed potential zone of inhibition against pathogenic bacteria *Bacillus subtilis* than *Escherichia coli* compared with antibacterial drug streptomycin. Therefor silver nanoparticles have the potential to serve as an alternative to antibiotics and to control microbial infections such as those caused by multidrug resistant pathogens.

Keywords: antibacterial effects, silver nanoparticles (AgNPs), Agar well diffusion, antibiotics, zone of inhibition.

Introduction:

Generally, in nanotechnology nanometer range particles are synthesized, characterized and applied in materials. Properties of nanoparticles are different from bulk properties and the difference is high surface area and very small size. Due to the low concentration they are non-toxic to the human body. Nowadays bacteria cause many infectious diseases which spread in our

environment which directly influence on human health. Many antibacterial drugs are available in the market to cure these bacterial diseases. The effect of drugs is limited which causes side effects on human health. On other handsome bacteria have develop multidrug resistance ability so they resist the effect of drug. Therefore, there is need and challengeto find out the substitute drugs which are strongly effective and harmless. So, many researchers started finding new potential antibacterial agents [1] according to them silver metal nanoparticles have received special attention as a possible antibacterial agent [2-5]. It has been known that silver and its compounds have strong inhibitory and bactericidal effects and antimicrobial activities for bacteria, fungi and viruses since ancient times [2, 6]. The toxic effects of silver on bacteria have been investigated for more than 60 years [7].Silver nanoparticles represent a class of materials with potential application involving catalysis, drug delivery and antibacterial activity [8-11, 2, 12-14]. An additional advantage associated with the use of eco-friendly methods applied in the production of Ag NPs in large scale [15-17].

Materials and Methods:

Collection of materials

A. flavus, A. fumigatus and *A. niger* were isolated from infected fruits and maintained on potato dextrose agar (PDA) medium at 28°C. The isolated fungi were identified by lacto phenol cotton blue mounting by morphological and microscopic observation. Clinically isolated gram positive *Bacillus subtilis* and gram negative *Escherichia coli* were used for check the antibacterial activity.

Biosynthesis of AgNPs

For the synthesis of silver nanoparticles, the biomass of each fungus were prepared by growing aerobically in glucose nitrate broth containing glucose-10 gm, KNo3-2.5 gm, KH2Po4-1 gm, MgSo4-0.5 gm and 1000 ml distilled water. The inoculated flasks were incubated on orbital shaker at 30°C and agitated at 120 rpm for 96 hr. The biomass was harvested after six days incubation by filtering followed by repeated washing with distilled water to remove any medium component from the biomass. In a 250 mL Erlenmeyer flaskfive g (wet weight) was brought in contact with 100 mL of double distilled water for 3 daysat 30°C and agitated again at 120 rpm. The cell filtrate was obtained by filtering it through Whatman filter paper No. 1. The 5 mL filtrate was treated with 5 mL of 1 mM AgNo3 solution in an Erlenmeyer flask and incubated at room temperature in dark. Control containing cell-free filtratewithout silver nitrate solution was run simultaneously as standard with the experimental flask. All experiments were done in three replicates.

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Characterization of AgNPs

UV-visible spectroscopy analysis

Change in color of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. Silver ion bio-reduction was monitored by sampling of aliquots (1 mL) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer (Cystronics UV-Vis spectrophometer 117). UV-Visible analysisof several weeks old samples was also carried out to check the stability of synthesized AgNPs.

Transmission electron microscope (TEM)

For TEMmeasurements, a drop of synthesized AgNPs was placed on the carbon coated copper grids and kept for dry.After dryness of sample grid loaded onto aspecimen holder. TEM micrographs of the sample were taken using the Morgagni 268D TEM instrument (AIIMS, New Delhi).

Antimicrobial activity

The antimicrobial activity ofthe synthesized AgNPs was assessed against twotests micro-organisms, viz., *Bacillus subtilis* and *Escherichia coli*. The overnightgrown test bacterial cultures were plated on Glucose nutrient agar. Four wells were cut on the plates using 4 cm width corkborer and 50 µL of AgNPs solution was immersed in three well while as in the 4^{th} well antibiotic streptomycin drug was dispensed with the help of micropipette. The plates were incubated overnight at 37°C for 24 hr, and observed for the presence of zones of inhibition.

Result:

Biosynthesis of AgNPs

When cell-free filtrates of three fungal isolates were incubated with silver nitrate salt, the color of cell filtrates were exhibited a gradual change to brown colorunder dark conditions. The color of the culture filtrate with silver nitrate salt changed to intense brownafter 24 hr of incubation whereas the control (withoutsilver nitrate salt) did not exhibit any color change (Fig-1).The color changes observed can be attributed to the surface plasmon resonance of deposited AgNPs [18].

Characterization of AgNPs

The UV-visible spectra of fungal cell filtrate of *A. flavus, A. fumigatus* and *A. niger* treated with the silver nitrate solutions showed a characteristic surface plasmon absorption band at 450, 400, and 450 nm respectively, and the maximum color intensity was obtained after three days.

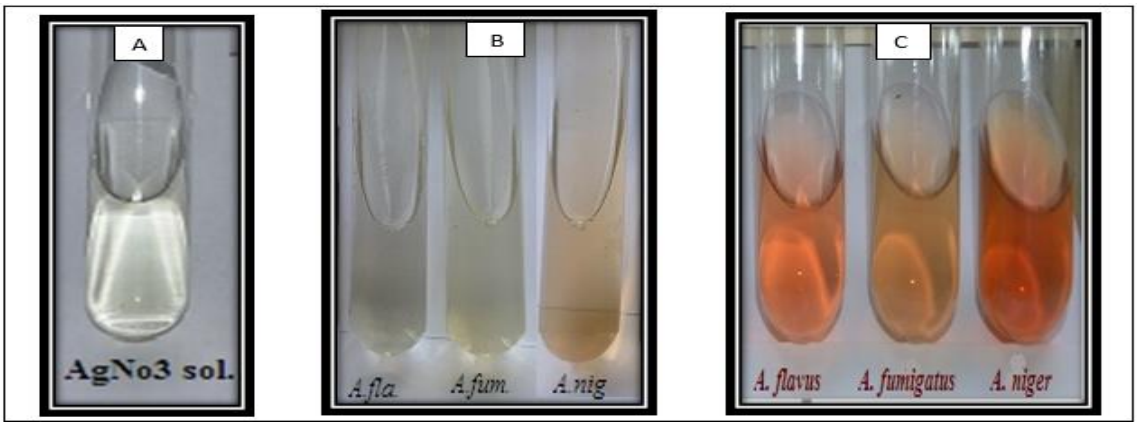

Figure 1: Color of sample A) 1 mM Silver nitrate solution B) Color of cell free filtrate of *Aspergillus* sps. before immersion of AgNo3 C) Color of cell free filtrate of *Aspergillus* sps. after immersion of AgNo3

Beyond three days of incubation, no further increase in intensity was recorded indicating complete reduction of silver ions by the fungal cell filtrate. Synthesized AgNPs was extremely stable at room temperature, without agglomeration after 60 days was monitored regularly byUV-visible spectrophotometer. This indicated that the nanoparticles were well dispersed in the solution without aggregation (Fig. 2).

TEM micrograph provided detailed morphology of silver nanoparticles. The data obtained from micrograph images showed distinct shape and size of polydisperse nanoparticles. Mostly particles were spherical but some are ellipsoidal in shape in the range of 9-60 nm without significant agglomeration (Fig. 3).

Antimicrobial activity

The fungus synthesized AgNPs were tested against *Bacillus subtilis* and *Escherichia coli* for the antimicrobial efficacy which resulted in formation of varying zone of inhibitions compared withinhibition zone of antibiotic streptomycin. The diameter of inhibition zone of AgNPs against *Bacillus subtilis* showed significant increase as compared to streptomycin than *Escherichia coli* (Table 1). *A. flavus* showed highest zone of inhibition than *A.fumigatus and A. niger* in both *Bacillus subtilis* and *Escherichia coli* (Fig 4)

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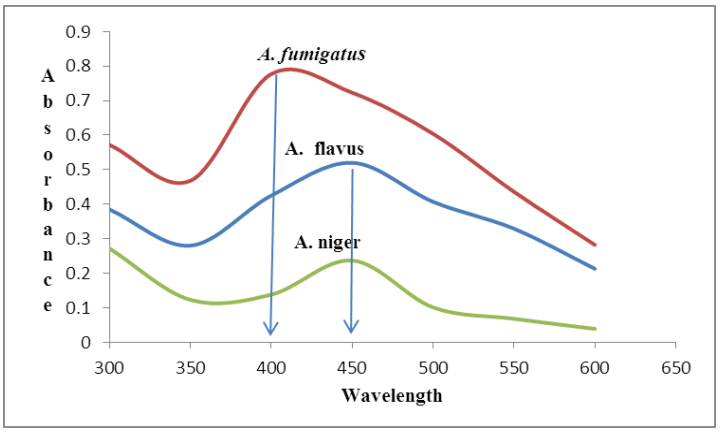

Figure 2: UV-Vis spectra recorded after the exposure of 1mM silver nitrate solution in crude cell filtrate of *Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus niger*

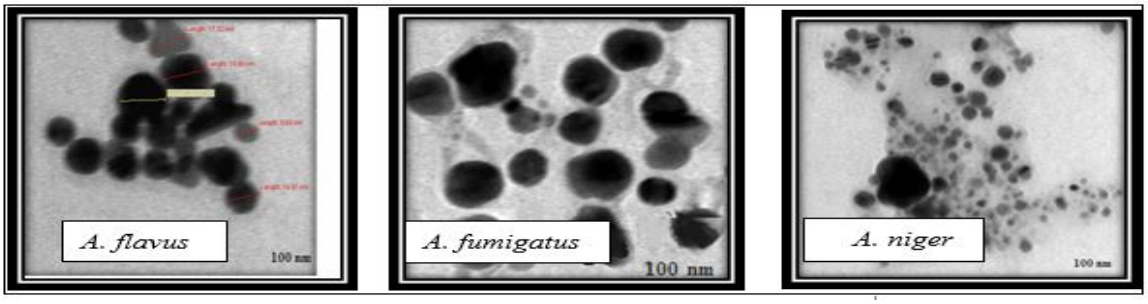

Figure 3: TEM micrograph of silver nanoparticles synthesized by *Aspergillus* sps

Table 1: Antimicrobial activity observed as zones of inhibition (mm) produced by mycosynthesized silver nanoparticles (AgNPs) against the test organisms

Silver nanoparticles of fungus	Zone of inhibition in diameter (cm)	
	Bacillus subtilis	***Escherichia coli***
A. flavus	*2*	1.8
A. fumigatus	1.8	1.6
A. niger	1.7	1.5
Streptomycin	1.4	2.1

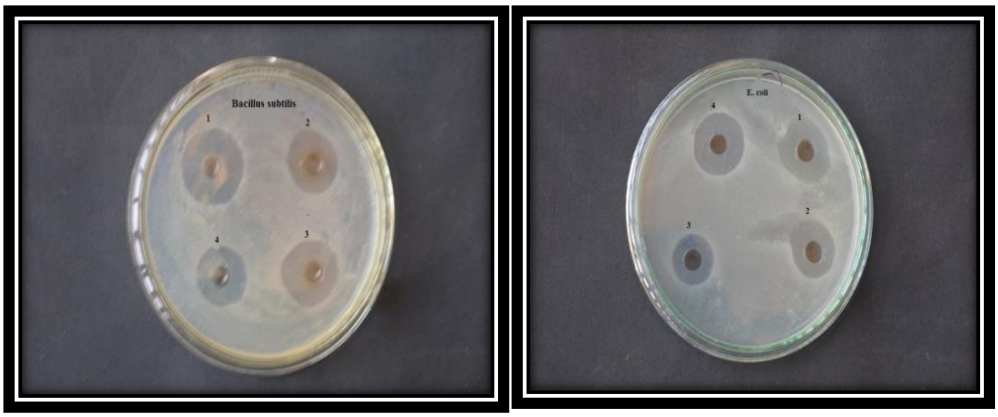

Figure 4: Antibacterial activity of silver nanoparticles against (A) *B. subtilis* (B) *E. coli* with 1) *A. flavus* 2) *A. fumigatus* 3) *A. niger* 4) streptomycin antibiotics

Summary:

The three species of *Aspergillus* genus observed absorbance peak at 450, 400 and 450 nm which are nearby similar to result of Ingle and co-worker [19], indicating the synthesis of silver nanoparticles by the selected fungal isolates characterized from infected fruits. TEM provided confirmation of presence of silver nanoparticles with detailed size and shape which are similar to the result of Jain and co-worker [20] using *A. flavus*. Silver is known to have broad-spectrum antimicrobialactivity against bacteria, viruses and eukaryotic microorganisms [21-22]. Silver nanoparticles observed effective antimicrobial activity against *E. coli* and *S.aureus* reported by Kim and co-worker [1].Our result is similar to that i.e. mycosynthesized silver nanoparticles have strong antibacterial properties than antibiotics against gram positive bacteria *Bacillus subtilis* and gram negative bacteria*Escherichia coli*.This study demonstrates that the mycosynthesized AgNPs showed potentantimicrobial activity against various pathogenic bacteria's namely, *Bacillus subtilis* and *Escherichia coli.*

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REVIEW OF THE PREVALENCE OF 'HEAVY METALS' IN FOOD MATRICES AT THE GLOBAL LEVEL

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Abstract:

The term "heavy metal" implies to the metals which contaminate the food chain and cause human toxicity even in the minute quantity. Among all heavy metals Lead, cadmium and arsenic are most toxic compounds based on the frequency of their occurrence in the environment and potential for human exposure. Heavy metals are widely present in the environment and contaminate the food chain. The presence of heavy metals is detected in various food matrices. Heavy metals toxicity can cause neurological damage, suppress the immune system and disrupt the endocrine system. In this perspective, the current review is meant to compile the reported data related to the existence of heavy metals in food products at the global level. Additionally, reports on the prevalence of heavy metals in the Indian scenario are also bringing together. This article reviewed the outcomes of numerous studies done worldwide on different types of food products. The obtained result shows that most of the food samples were contaminated with a different range of contaminants depending on the location of collection and detection method. In order to lessen its human exposure and reduce its health impacts, more research and awareness are needed in this field.

Keywords: Environmental pollutant, Heavy metal, Food products, Lead and Cadmium

Introduction:

Environmental heavy metals are the non-essential element that is neither created nor biodegradable and originates itself (Barbier *et al.*, 2005). The Agency for Toxic Substances and Diseases Registry has ranked heavy metals among the most hazardous and toxic substances in the environment (ATSDR, 2007). Among all heavy metals, arsenic, mercury, chromium, lead, and cadmium are ranked the highest among toxic metals, and amongst them lead is the most common heavy metal present in foodstuffs. Lead is listed in the substance priority list (SPL) as the second-highest toxic substance, due to its persistent occurrence and accumulation in the environment and subsequent human exposure. These heavy metals accumulate in the

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environment and contaminate the food chain (WHO, 2010). Heavy metals can be released into the environment by both natural and anthropogenic activities. However, in this era of industrialization and urbanization on earth, human activities are mostly responsible for the dispersal of heavy metals into the environment (Nagajyoti *et al*., 2010). Discharge of wastewaters such as industrial effluents and domestic sewage, application of inorganic fertilizers which may leach into the groundwater has led to the contamination of agricultural soils (Rey-Crespo et *al.*, 2013). It accumulates in the soil to contaminate the food chains and cause a potential health threat to their consumers including humans. Milk is one of the most significant parts of the diet for a significant proportion of the global population. It is believed that milk and dairy products are the principal foodstuffs acting as a source of exposure to humans (Suturović *et al.*, 2014; Nejatolahi *et al.*, 2014; Oliveira *et al*., 2017; *et al.*, 2017). In addition, heavy metals can accumulate in various other food matrices such as soft drinks, fruits, frozen products during their industrial processing and cereals and grains (Izah *et al.*, 2017). Further, heavy metals adversely affects human reproductive health causing miscarriage, infertility, premature delivery, and pre-eclampsia in females and abnormal spermatogenesis, chromosomal change, reduction in libido, and infertility in males (Saleh *et al.*, 2009;Assi *et al.*,2016). It also causes anemia, kidney, and brain-related problem (Sharma *et al.*, 2014; ATSDR, 2007). In this background, this review shows the presence of Pb in food products at a global level, to aware the public about their occurrence.

Heavy metals:

Heavy metals are defined as “metal" with a density greater than 5 g/cm^3 (Csuros and Csuros, 2002). These can be classified as trace essential, precious, radio nuclei, and highly toxic metals. Essential heavy metals like copper, iron, zinc, selenium, and cobalt have an important role in various biological processes in humans (Mertz, 1981). However, the term “heavy metal” implies to the metals which contaminate the food and cause toxicity even in minute quantity (Duffus, 2002). It includes heavy metals such as lead, mercury, arsenic, and cadmium which are highly dispersed in the environment and because of their usages in various industrial processes like metal plating, textile, battery manufacturing, metallurgy, chemical manufacturing, automobiles, and petroleum (Arruti, 2010, Sträter *et al.*, 2010, Pacyna, 1996). These environmentally toxic heavy metals have a very long half-life and persistency in nature. Their exposure to humans can cause many life-threatening problems including cancer, cardiovascular diseases, skin lesions, memory problems, increased heart rate, tremors, spontaneous abortion, nervous system damage, major organ damage, and death (ATSDR, 2007). Therefore, the United States environmental protection agency (VEPA) registered heavy metals as the major pollutants.

Based on toxicity, frequency of occurrence, and potential for human exposure, the US Agency for toxic substance and disease registry (Abadin et al., 2019) ranked heavy metals most toxic substance in the substance priority list (SPL). Among all metals lead (Pb) is most toxic has various important Physico-chemical properties due to which it is widely used in industrial processes (Gabby, 2003). Additionally, it is also used in some fertilizers and pesticides for agricultural purposes (Hernberg, 2000). Furthermore, its properties like non-biodegradability, bio-magnification, the long half-life, and persistency in nature make it a more potent toxin. Heavy metals half-life is different in different body parts. For instance, half-life of lead in different parts of the body is varies from days to years (blood -25-36 days, muscle -40 days, bone -20 years)

Route of exposure of heavy metals:

Lead is an extensively used heavy metal in several industries such as metal plating, textile, battery manufacturer, and automotive especially in gasoline (Klaassen, 2006). Similarly, other heavy metals are also used in various industrial processes. These metals can enter into the agricultural fields through various ways such as direct discharge of industrial wastages and sewage discharge from the industries containing heavy metals (Ziarati *et al*., 2018), falling of rain contaminated with lead, arsenic(As) and cadmium (Cd), application of lead-containing fertilizers (Younus *et al.,* 2016). From contaminated agriculture fields, these toxic metals reach to the crops and contaminate the whole food cycle. Additionally, heavy metals can accumulate in various beverages like juices, soft drinks and canned food products during their industrial production. In addition to it, dairy animals are exposed to heavy metals by the consumption of contaminated feed and drinking water (Amponsah, 2014). It accumulates in the tissues and bones of dairy animals due to its stable structure and subsequently excreted into the milk (Burger and Elbin, 2015). Lead can also be introduced into milk during milk processing and transportation (Anetta, 2012).

Toxic effects of heavy metals:

Heavy metals are widely present in the environment as a persistent pollutant that poses a constant hazard to mankind. Its contamination is dramatically increasing mostly in developing countries particularly in India and China (Rai *et al.,* 2019). Exposure to heavy metals can cause many problems related to reproduction in humans as well in animals. In males, heavy metals toxicity can cause abnormal spermatogenesis, chromosomal change, reduction in libido, and infertility (Assi, *et al.,* 2016). On the other hand, heavy metal toxicity can disrupt the steroid genesis process, and lead to miscarriage, infertility, premature delivery, and pre-eclampsia in females. Lead negatively affects ovarian fetus development (Saleh *et al.,* 2009). In addition to it,

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lead can also induce anemia by targeting three main enzymes of heme biosynthesis i.e. ALA dehydratase (ALAD), uroporphyrinogen decarboxylase, protoporphyrinogen oxidase, and ferrochelatase (FeC) (Sharma *et al.,* 2014). Lead toxic effects can be seen on major organs such as kidney nephrotoxicity, brain neurotoxicity, and heart problems (ATSDR, 2007). Hormones such as T3, T4, IGF-1, ERβ, LH, FSH, progesterone, and testosterone are also affected by lead (Taupeau *et al.,* 1982; Kolesarova *et al*., 2010). On the other hand cadmium and arsenic are well known carcinogenic compounds. Cadmium is involved in the etiology of prostate cancer arsenic known to cause skin cancer (Kolluru *et al*., 2019; Eryani *et al*., 2018; Saran *et al.,* 2021). Heavy metals effects on human health are summarized below in Table 1.

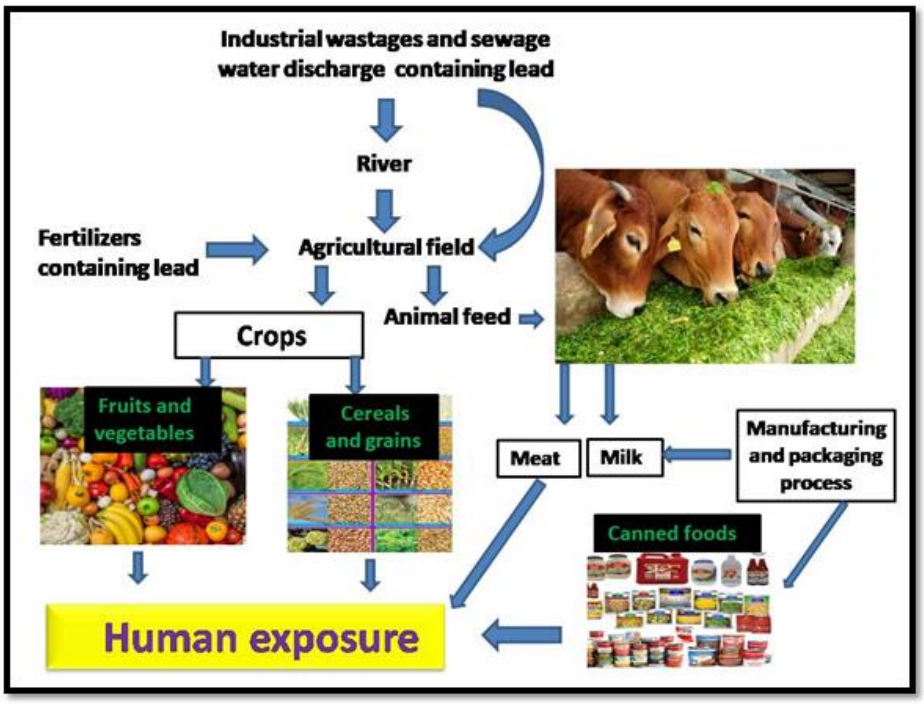

Figure 1: Route of exposure of heavy metals to human

Table 1: Human health effects of heavy metals

Body Systems	Toxic effect	References
Reproductive Effects	Infertility in male and female and miscarriages	ASTDR, 2007
Endocrine Effects	Disruptor of adrenal and ovarian steroidogenesis	Taupeau *et al.*,1988
Neurological Effects	Brain damage, disruption of nervous systems and intellectual disorders	Chibowska *et al.*, 2017
Hematological Effects	Affects the membrane stability of erythrocytes and anemia (less Hb)	Goyer and Rhyne, 1973
Cardiovascular Effects	Hypertension and elevated blood pressure	Vaziri and Gonick, 2008
Carcinogenic Effects	Prostate and skin cancer	Kolluru *et al*., 2019; Eryani *et al*., 2018

Worldwide status of heavy metals in the food matrices:

Heavy metals are very toxic, concerning the toxicity of most toxic heavy metal lead it is banned by the US in the most consuming gasoline industry (EPA, 1975). Various other organizations are also spreading awareness about the crucial effects of heavy metals on living organisms and actively promoting their restricted uses in the various industries (Suk, 2002). However, after so many efforts presence of these chemicals in the food matrices cannot be neglected. The worldwide status of heavy metals in the foodstuffs has been summarized in the form of table which is given below (Table.2).

Status of heavy metals in India:

Few studies have been conducted regarding the presence of heavy metals residues in food samples of India and these studies reported the occurrence of different heavy metals in different kinds of food samples (Chandrakar *et al 20*18; Kambli *et al.,* 2019).In a previous study, (Zodape *et al.,* 2012) studied the cow milk samples of different brands from a different region of Mumbai by using the ICP-MS method and observed that all 15 milk samples were contaminated with lead in a range of 0.139- 5.904 µg/mL. This detected concentration of lead is much higher than the maximum permissible limit. Similarly, in another study, effect of location was studied on the level of contaminants in the milk samples. For this, Swarup *and coworkers* collected 149 milk samples from lead-zinc smelter rich area of India, and 52 samples from the non-contaminated area, they analyzed both different types of samples comparatively using AAS and found 0.84+/-0.11µg/mL concentration of lead in the milk sample obtained from lead-zinc- smelting area. It reflects the geographical effect on the occurrence of heavy metals (Swarup *et al.,* 2005). Furthermore, it was reported that lead contamination is more in buffalo's milk as compared to cow's milk (Iqbal *et al*., 2011). Table 3 given below provides an overview of the heavy metals contamination in milk and other food products in the Indian scenario.

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Table 2: The worldwide status of Heavy metals in the Food matrices

Element	Country	Food product	Remarks	References
As and Cd	Peru	Grains and processed products	Polished rice and quinoa products were heavily contaminated	Roman-Ochea *et al.*, 2021
As, Hg, and Pb	Italy	Seafoods, Beefs and Pork meats	All heavy metals were contaminated. Cd and Pb exceeded the MRL value	Bella *et al.*, 2020
Cd, Pb, Cu and Zn	China	Wheat grains	Contamination of wheat grain samples due to presence of lead smelter region	Li *et al.*, 2019
Pb	Ghana	Vegetables	Hazards index for all metals were below detection limit except for cadmium	Ametepey *et al.*, 2018
Pb, As, Cd, and Hg	Tehran	Fruits, juices and canned products	All 72 samples were contaminated and 36 samples were above the codex limit	Fatahabad *et al.*, 2018
Cd and Pb	Nigeria	Beverages (soft drinks, candies, chocolates)	All samples were contaminated	Izah *et al.*, 2017
Pb and Ni	Italy	Pre-packed coffee products	Detected in all coffee samples	Toni *et al.*, 2017
Cd, Pb, As, and Hg,	Iran	Milk	Pb in the samples was higher than the European Union and Iran's national standard	Rezaei *et al.*,2014
Cd, Pb and Cu	Serbia	Milk and fermented products	Two samples were contaminated with Cd and Pb	Suturović *et al.*, 2014
Co, Cr, Cu, Ni, Zn, As, Cd, Hg and Pb	Spain	Organic and conventional milk samples	Toxic metal in milk were in general very low and no statistically significant. Differences were observed between organic and conventional milk	Crespo *et al*., 2013
Pb and Cd	Nigeria	Milk	All samples have lead more than MRL value	Tona *et al.*,2013
Zn, Pb, Cr, Ni, Cu, As, and Cd	Italy	Milk and dairy products	Lead levels were below the maximum limits as set by the EC in almost all samples tested.	Licata *et al.*, 2012

***All heavy metals symbols are explained as:** As- Arsenic, Cd- Cadmium, Cr- Chromium, Co- Cobalt, Cu- Copper, Hg- Mercury, Pb- Lead, Ni- Nickel, Zn- Zinc.

Table 3: Indian status of heavy metals in the food matrices

Element	States	Food product	Remarks	References
As	Bihar	Wheat	77 wheat flour samples tested, all were contaminated	Suman *et al.*, 2020
Cd, Zn, Ni & Cu	Karnataka	Cereals & vegetables	Cd was exceeding the toxic limit in some samples	Shobha & Kalashetty, 2017
Pb, Cd, Co, Cu, Cr & Zn	Haryana	Rice grains & paddy fields	All samples contaminated below the MRL limit	Yadav *et al.*, 2016
Pb , Cr & Ni	Maharashtra	Milk	Most of the samples contaminated	Nirgude *et al*., 2015
Pb & ZN	Telangana	Milk	All samples heavily contaminated with Pb	Shailaja *et al.*,2014
As, Cd, Pb & Hg	Tamil Naidu	Milk	High level of Pb and Cd was observed	Dhana-lakshami *et al.*, 2013
Pb	Gujarat	Milk	All samples were contaminated	Chandrokar *et al.*,2013
Pb & As	Delhi	Milk	All samples were contaminated	Raina *et al*.,2013
Pb, Hg & Cr	Maharashtra	Milk	Highly contaminated with lead	Zodape *et al* .,2012
Pb	Tamilnaidu	Milk	All milk samples were safe	Sahayara & Ayyadurai, 2009
Zn & Cd	Karnataka	Milk	Heavily contaminated with non toxic Zinc	Lokeskeshwari & Chandrappa.,2006
Pb & Cu	Maharashtra	Milk	Contaminated with toxic lead	Swarup *et al.*,2005
Pb	Various part of India	Milk	State specific contamination was observed	Tripathi *et al.*,1999

All heavy metals symbols are explained as: *As- Arsenic, Cd- Cadmium, Cr- Chromium, Co- Cobalt, Cu- Copper, Hg- Mercury, Pb- Lead, Ni- Nickel, Zn- Zinc.

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Conclusion:

The prevalence of heavy metals in food products varies greatly from country to country and also within the country. This variation can be correlated with various factors, such as the location of the sample collection site, types of sample, methods of detection, and government policies. So, people should be aware of their occurrence in the food products and toxic effects on humans. Further, research on the toxic effect of heavy metals with their mode of action in humans should be encouraged. Globally from the government to the public, researchers to industrialists' presence of these heavy metals in food matrices should be addressed to mitigate their health impacts. This study concludes by suggesting that heavy metal concentration in the foodstuffs should be monitored by producers, and its concentration in industrial food products should also be monitored by appropriate regulations.

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STUDY THE *IN VITRO* DETERMINATION OF V_{max} AND K_m IN ENZYME ACTIVITY

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Abstract:

The in vitro determination (in vitro = within the laboratory) of the kinetic parameters has been accomplished through certain methodologies that have been examined. The experiment enzyme activity using alpha-amylase is carried out for finding the values of the kinetic constants. The method to be applied is also mentioned clearly. The simple and easy process of determination is made via this experiment. Moreover, the requirements are cost-efficient and many trials are performed for getting actual values. Readings obtained from the observations of the experiment enabled to plot of the graph using methodology. The results gained are almost accurate since the method applied is highly productive. The readers who go through it will gain a perception regarding the kinetic parameters in-depth.

Keywords: Kinetic Parameters, Value determination, Lineweaver - Burk Plot

Introduction:

In natural chemistry, Michaelis–Menten energy is outstanding amongst other known models of compound energy. It is named after German organic chemist Leonor Michaelis and Canadian doctor Maud Menten.The model appears as a condition portraying the pace of enzymatic responses, by relating response rate, the grouping of a substrate S. Its equation is given by

$$Vo = Vmax . [S]/Km + [S]$$

This condition is known as the Michaelis–Menten condition.Vmax is a volumetric rate that is relative to the measure of dynamic catalyst present. The Michaelis steady Km is equivalent to the reactant focus at which rA=Vmax/2. Km is free of protein fixation yet shifts starting with one compound then onto the next and with various substrates for a similar catalyst.

Here, Vmax addresses the greatest rate accomplished by the framework, occurring at soaking substrate focus. The worth of the Michaelis consistent Km is mathematically equivalent to the substrate fixation at which the response rate is half of Vmax. Biochemical responses including a solitary substrate are regularly accepted to follow Michaelis–Menten energy, regardless of the model's basic presumptions.Precise upsides of Vmax can be resolved based on Lineweaver – Burk condition, which is determined by taking the proportional of Michaelis - menten condition.The Lineweaver-Burk condition is a straight condition, where 1/V is a direct capacity of 1/[S] rather than V being an objective capacity of [S]. The Lineweaver-Burk condition can be promptly addressed graphically to decide the upsides of Km and Vmax.

Background:

In 1901, French actual physicist Victor Henri found that chemical responses were started by a security (all the more by and large, a limiting association) between the compound and the substrate. His work was taken up by German natural chemist Leonor Michaelis and Canadian doctor Maud Menten, who explored the energy of an enzymatic response instrument, invertase thatcatalyzes the hydrolysis of sucrose into glucose and fructose. In 1913, they proposed a numerical model of the response. It's anything but a chemical, E, restricting to a substrate, S, to frame an unpredictable, ES, which thusly delivers an item, P, recovering the first compound. This might be addressed schematically as

$$E + S \rightarrow P + E$$

Where E is compound, S is substrate, and P is item. ES is the chemical substrate complex. True to form in synergist responses, compound E is recuperated toward the finish of the response. Restricting of substrate to the compound in the initial step is viewed as reversible with forward response steady k1 and opposite response consistent k−1. Deterioration of the catalyst substrate complex to give the item is an irreversible response with rate consistent k2; k2 is referred to as the turnover number as it characterizes the quantity of substrate atoms changed over to item per unit time by a chemical soaked with substrate. The turnover number is in some cases alluded to as the reactant steady kcat.

Methodologies of technology:

The Michael Menton plotting method plays an important role in the determination of Km and Vmax in usual. Though this method is highly effective, there are various methods for nicer determination of those two kinetic parameters (Km, Vmax) in graphical presentation. Also, there is another method derived from the reciprocal of the Michel Menton equation called the

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Lineweaver - Burk equation method. Due to the plots 1/ [S] versus 1/ Vo, the Lineweaver - Burk plotting method is often referred to in the name of the double reciprocal plotting method. This method is fundamental for obvious representation for graphs

$$\text{Starting rate of reaction, } Vo = \frac{Vmax \times [S]}{Km + [S]}$$

Here Vmax is the maximum rate of enzyme reaction, Km is the Michael-Menton constant, [S] is the substrate. According to the Michael - Mendel theory assumption, the enzyme [E] initially tries to assemble with the substrate [S] for the formation of the Enzyme -Substrate complex.

Materials required:

- 0.1 % Starch
- Dinitrosalicylic acid (DNSA) reagent
- 0.1 % Alpha amylase
- Phosphate buffer solution (pH value = 7)
- Test tubes, Beaker, Heat flame(for boiling)

Figure 1: Phosphate buffer, Alpha-Amylase, Starch

Determination of alpha-amylase:

The α -amylase activity is measured using a colorimetric method withinto maltose. Maltose released from starch is measured by the reduction of 3,5-dinitrosalicylic acid.α-Amylase is presumably the catalyst with the most distributed techniques for measure of its reactant action .Early systems utilized as substrate starch, amylose, amylopectin, or some artificially adjusted

subsidiaries of polymers. More re-cently, maltooligosaccharides of characterized chain length have been presented as substrates for a-amylase, and particularly those having a 4-nitrophenyl or 2-chloro-4-nitrophenyl bunch appended to the decreasing finish of the chain are presently being used. In these strategies, α -amylase parts the substrate into pieces, which are thusly hydrolyzed by the helper chemical α -glucosidaseto more modest sections, glucose, and free chromophore. A further improvement in strategy for deciding α -amylase was the presentation of 4-nitrophenyl oligosaccharides synthetically obstructed at the nonreducing end. The presence of the hindering bunch permits the utilization of glucoamylaseas a helper protein, notwithstanding α–glucosidase.

Observation:

S.No	0.1% of Starch Solution	Phosphate buffer (in ml)	Substrate concentration[S]	Alpha - amylase (in ml)	1/(S)
0	-	-	-	1 ml	-
1	0.1 ml	1.0 ml	100 ug/ml	1 ml	0.01
2	0.2 ml	0.9 ml	200 ug/ml	1 ml	0.005
3	0.3 ml	0.8 ml	300 ug/ml	1 ml	0.0033
4	0.4 ml	0.7 ml	400 ug/ml	1 ml	0.0025
5	0.5 ml	0.6 ml	500 ug/ml	1 ml	0.002
6	0.6 ml	0.5 ml	600 ug/ml	1 ml	0.00167
7	0.7 ml	0.4 ml	700 ug/ml	1 ml	0.00143
8	0.8 ml	0.3 ml	800 ug/ml	1 ml	0.00125
9	0.9 ml	0.2 ml	900 ug/ml	1 ml	0.0011
10	1.0 ml	0.1 ml	1000 ug/ml	1 ml	0.001

After an incubation at room temperature for 10 min and then added DNSA. Again an incubation boiling water bath for 10 min

DNSA	OD	OD/slope	EA	1/Vo
1	0	-	-	-
1	0.57	60.39	17.66	0.056
1	0.157	164.57	48.12	0.02
1	0.321	335.29	98.03	0.0102
1	0.381	397.64	116.27	0.0086
1	0.48	501.03	146.51	0.0068
1	0.538	560.64	163.93	0.0061
1	0.631	657.66	192.3	0.0052
1	0.683	712.48	208.33	0.0048
1	0.73	761.42	222.64	0.0044
1	0.795	829.14	242.44	0.0041

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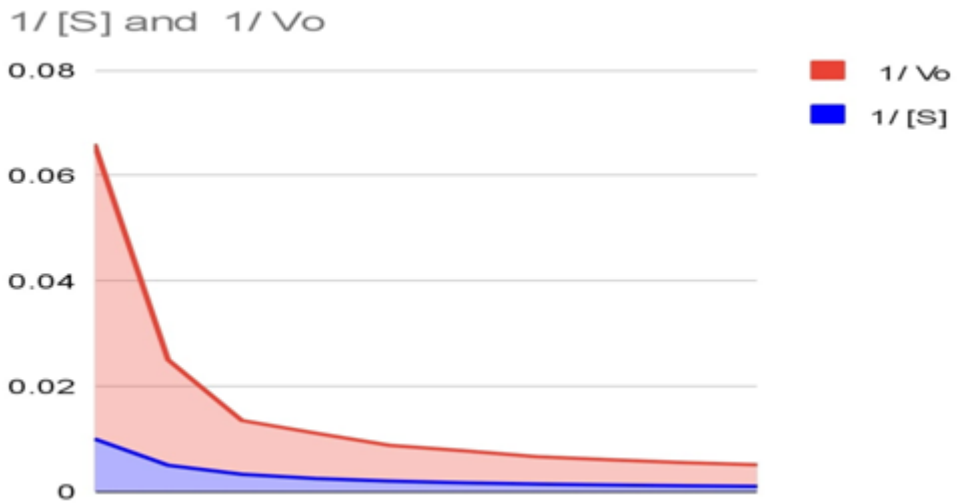
Graph:



Results and Discussion:

By using the perfect methodology (i.e., the double reciprocal plot) for the in vitro determination of the kinetic parameters (Kmand Vmax) of the enzyme (α-amylase) taken, the results are achieved precisely by detailed readings assumed for consideration. Therefore the values are estimated as follows, Vmax = 769.23 umol/min; Km = 384.615 ug/ml.[Note: The range of both values persists the same when any two points from the plotted graph are taken for slope]. Hence, the result is obtained by the double reciprocal plot / Lineweaver - Burk plot. This verifies that the method chosen is for better determination of the parameters.

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CESTODES: SERIOUS HEALTH CONCERN

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Parasite diseases are among the major public health problems of tropical countries including India. They infect man and also severally invade domestic animals and wild life. The vertebrates are one of the components of food cycle. As the Cestodes are common occurrence in the Vertebrates, from the class Pisces to class Mammals, they may be transferred in the body of man, due to which many diseases are caused.

The cestode parasites, present in these vertebrates hosts, cause serious diseases, when man eats the under cooked flesh is improproply cooked and is consumed by the Human being the parasites when present, increase the rate of mortality of hosts, reduce the rate of reproduction and also reduce their food value.

So far, little work has been done, on the cestode parasites of vertebrates in India. Eminent personalities realized the importance of systematic, taxonomy and morphology of cestode parasites. Cestodes are dorsiventral flattened, acoelomate, triploblastic, bilaterally symmetrical, hermaphrodites, free living or parasitic worms, adhesive organs like hooks, spine, suckers and adhesive secretions are present. Taenia solium is common example of human parasite. It completes its life cycle in two hosts, namely man and pig. Man is primary host and pig is the secondary host.

Infection to Pig: When a pig eats the faces, the onchospere enters the intestine of the pig. Here the embryonic membrane dissolves. The larva penetrates the intestinal wall with the help of hooks. The larva then reaches the muscles of the pig through the arterial system. In the muscles it loses the hooks and develops into the next larva called cysticercus or bladder worm.

Infection to man: When man eats the under cooked flesh of pig, cysticercus enters the intestine. The covering dissolves. The Scolex is attached to the intestinal wall. The neck produces proglottids. The bladder becomes detached and disintegrated. An adult tapeworm is formed in a few weeks.

The body of the Taenia solium is divided into three regions, namely the Scolex, the neck and strobila. The Scolex is a small knob like structure, situated at the anterior end. It has the size of a pin-head. It possesses a small terminal cone called rostellum. Around the base of the rostellum, there is a double circle of chitinous hooks. The Scolex has four suckers, which project from the surface. The suckers and hooks help to attach the animal to intestine of the host. The tapeworm is a segmented animal showing a repetition of structure in each segment. Each proglottids contains a set of male and female reproductive organ and a part of the nerves and excretory systems. But the youngest segments lying near the neck are having no sex organs. They are termed as immature proglottids. The proglottids of the middle region are termed as mature proglottids which contain sex organs. The posterior most segments are called gravid or ripe proglottids which contains fertilized eggs.

Taenia solium scolex consists of rostellum, hooks and sucker. Mature proglottids consists male and female reproductive system. The male reproductivesystem consists of numerous small s, genital atrium, rounded testes, vas deferens, genital atrium, genital pore and last part muscular cirrus and cirrus sac. The female reproductive system consists of a single bilobed ovary, oviduct, vagina, uterus, seminal receptacle, female genital pore, Mehlis glands and ootype. Gravid segment consists fertilized eggs with eggs capsule.

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DISPARATE VARIATION OF GENETIC DISORDERS AND SYSTEMIC STRUCTURAL ANATOMY OF EYE

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Genetic Disorders

Genetic disorders are a category of diseases triggered by any alteration in gene called mutation. These mutations can transpire randomly or because of environmental factors. These abnormalities can extend the range from a point mutation in a single gene or, deletion/duplication, of an entire chromosome or set of chromosomes. These genetic disorders are inherited as single gene, chromosomal, mitochondrial and complex diseases [Goss, 2014].

Keywords: Genetic disorder, eye, complex disorder, retina

Single Gene Disorder

A single mutation in a gene might emerge into a single gene disorder. Atop of almost 4000 human diseases wereoriginated due to single gene defects. Single gene disorders can be passed on to subsequent generations in several ways as a dominant or recessive trait [Huang and Keiles, 2012].

Autosomal Dominant Disorders

Autosomal dominant is one of the ways that a trait or disorder of a gene is inherited in a dominant condition, where only one mutated allele is adequate to express the disease. The person affected with autosomal dominant disorder has one copy of a mutant gene. In autosomal dominant disorder usually one of the parents is affected and transmits the disease to 50% of their offspring [Bateman *et al.*, 2011]. Example: Huntington's disease [Nopoulos, 2016], Marfan's syndrome [Yuan and Jing, 2010]. Marfan's syndrome is a genetic disorder of the connective tissue and inherited as a dominant trait, carried by the gene FBN1, which encodes the connective

protein fibrillin-1. This disorder is characterized by long limbs and long, thin fingers, with most consequential complications of defects in heart valves and aorta. Marfan's syndrome can also seriously affect the eyes and vision. Nearsightedness and astigmatism and earlier glaucoma are frequent in these Marfan's syndrome patient. Also this has critical effect on cardiovascular system having the clinical symptoms like heart palpitations, racing heartbeats, or angina pectoris with pain radiating to the back, shoulder, or arm, and shortness of breath [Yuan and Jing, 2010].

Autosomal Recessive Disorder

Autosomal recessive is one of the ways that a trait or disorder of a gene is inherited in a recessive condition, where both the mutated allele contribute for the expression of the recessive character. In autosomal recessive disorder usually both the parents remains unaffected, but persistthe carrier of the single allele of the mutated gene. Example: Bardet-Biedl syndrome [Kara *et al.,* 2006]. Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder represented primarily by retinal pigmentary dystrophy, obesity, polydactyly, hypogenitalism, and mental retardation, and secondary symptoms such as renal dysplasia, diabetes mellitus, hearing loss, hepatic fibrosis, and speech impairment [Beales *et al.,* 1999].

X-Linked Dominant

X-linked dominant disorders are induced by mutations in genes on the X chromosome and inherited as a dominant trait. Both male and female are affected by this disorder but male have the severe effect than the females [Dobyns et al. , 2004]. Example: Danon's disease [Taylor *et al.* , 2020; D'Souza and Law, 2021]. Danon's disease is an X-linked dominant ocular disorder originated by mutations in LAMP2 gene. Clinical changes include pigmentary changes variously called a peripheral pigmentary retinopathy or a pigmentary atrophy [Taylor *et al.* , 2020; D'Souza and Law, 2021].

X-Linked Recessive Disorder

X-linked recessive disorders are effectuated by mutations in genes on the X chromosome and inherited as recessive trait. Males are very frequently prone to this disorder rather than female. Here a female carrier has 50% of her son affected and 50% of her daughter as carrier of the disorder. Example: Haemophilia A, Red-Green colour blindness [Dobyns et al. , 2004; Basta and Pandya, 2021]. Red-Green colour blindness is the inability or diminished ability to see color, or perceive color differences, under normal lighting conditions. This type of color blindness is generally a sex-linked condition. The color blindness inherited can be from birth (congenital) or it might commence during childhood or in the adulthood. Depending on the classification of

mutation, it might be stationary, that is, it persist the same throughout a person's lifetime, or progressive [Gordon, 1998; Wong, 2011].

Y- Linked Disorder

Y-linked disorders are generated due to mutations on the Y chromosome. Y-linked traits are passed from father to son, with no interchromosomal genetic recombination and the inherited pattern is called as holandric inheritance. In Y – Linked disorders the affected father has strained affected sons and unaffected daughters. Example: Retinitis pigmentosa, male sterility, azoospermiaetc [Kumar *et al.* , 2020; Lau, 2020]. Retinitis pigmentosais also triggered due to mutations in the RPY (retinitis pigmentosa, Y-linked) gene. It is characterized by declined night vision, followed by dropping or loss of peripheral or side vision and finally blindness [Pagon, 1988; Fahim, 2018].

Chromosomal Abnormalities

Chromosomal abnormalities are enkindled due to alteration in the normal structure or number translocation, inversion, insertion, deletion in a portion of the chromosome. And the abnormalities in chromosome number is induced due to monosomy, trisomy etc. Example: Down's syndrome [Bowen, 1964; Clendenin, 1966]. Down's syndrome, also known as trisomy 21, is a chromosomal condition created by the presence of a third copy of chromosome 21. It is characterized by stunted growth, mental retardation, brachycephaly, congenital heart disease, short neck, umbilical hernia etc. [Gath, 1994; Crawford and Dearmun, 2016; Sánchez Pérez, 2018].

Mitochondrial Disorders

Mitochondrial disorders are generated by the mitochondrial DNA (mt DNA), which affects mitochondria function. This is distinguished by poor growth, loss of muscle coordination, muscle weakness, visual problems, hearing problems, learning disabilities, heart disease, liver disease, kidney disease, gastrointestinal disorders, respiratory disorders, neurological problems. Mitochondrial DNA mutations occur frequently, due to the lack of "Proof Reading" ability, example: Leber's hereditary optic neuropathy (LHON) [Gorman *et al.*, 2016; Molnar and, Kovacs, 2017; Kisilevsky *et al.,* 2019]. Leber's hereditary optic neuropathy (LHON) is an eye disorder characterized by progressive loss of central vision due to degeneration of the optic nerves and retina [Manickam*et al.,* 2017; Priglinger *et al.,* 2019].

Multifactorial, Polygenic (or) Complex Disorder

Multifactorial or polygenic disorders are originated due to the close association and interaction of multiple genes in combination with environmental factors. This complex disorder

does not fit into the Mendelian inheritance pattern [Mitchell, 2012]. A complexdisease with a prevailing inheritable component is polygenic, meaning that several or diversedissimilar changes in DNA are the genetic foundation for the disease [Mellerup *et al.,* 2012]. These disorders are difficult to study, and also complicated to find the causes ex: Metabolic syndrome, cancer, diabetes, obesity, cardiovascular diseases, blood pressure, etc [Huang, 2009; Samson and Garber, 2014; McCracken *et al.,* 2017].

Parts of the Human Eye

Human eye is a photoreceptor organ, which is sensitive to light and photosensitive ganglion cells in the retina receive the light signals which adjusts the size of the pupil, regulation and suppression of the hormone melatonin. The human eye has many parts, which involve in divergent functions [Irsch andGuyton, 2009].

- Sclera - Sclera is the white wall of the eye and is flexible, tough and durable with numerous tendons attached to it and whose internal fluidic pressure keeps the eye in shape and protects its delicate internal parts [Boote *et al.,* 2020].
- Cornea - Cornea is a dome shaped outer covering which is a main refractive surface of the eye where most of the focusing of light occurs. It is transparent and thick which comprise of multiple layers of epithelium, highly supplied with nerve fibres. It is highly sensitive to foreign bodies, cold air, chemical irritation and tears, sustain and maintain oxygen exchange and water content and also prevent scattering and improve optical quality [DelMonte and Kim, 2011].
- Choroid - Choroid coat or choroidea is a vascular layer containing blood vessels and connective tissue situated between the retina and sclera which supplies blood and nourishment to the retina. The human choroid is of 0. 2 mm thickness and the outlying areas narrows to 0. 1 mm [Nickla and Wallman, 2009]
- Ciliary body –Interior circumferential tissue of the eye is termed ciliary body, which constitutes of ciliary muscle and ciliary processes with double layered ciliary epithelium. The main function of ciliary body is production of aqueous humor which provides nutrients and maintenance of the lens zonules. It also anchors the lens in place [Peces-Peña *et al.,* 2013; Miesfeld and Brown, 2019]
- Iris - Iris is the coloured part of the eye having ring of muscle fibres located behind the cornea and in front of the lens. The iris comprehend the sphincter pupillae, a muscle utilized to narrower the pupil and also operates to widen the pupil dilator called pupillaea muscle. The iris of the eye helps protect the sensitive retina [Neuhuber and Schrödl, 2011; Aguirre, 2019; Dorgaleleh *et al.,* 2020]

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- Pupil - The Pupil is the dark, black circle positioned in the centre of iris and allows light to enter the retina. It contracts with brightness and expands during darkness allowing light to better transmit [Aguirre *et al.*, 2019; Bouffard, 2019].
- Retina - The retina is the intramural interior most surface layer on the sensitive tissue. The retina is the film of the eye and it consists of many layers of rods and cones. It transforms the light rays into electrical, gesticulation signals and transmit them to the brain uninterrupted via the optic nerve. The retinal sides are accountable for peripheral vision. The center area, called the macula, is used for our fine central vision and colour vision [Hoshino *et al.*, 2017].
- Macula - The back center of the retina contains the macula measuring about 7 mm in diameter. One of the exceedingly sensitive segment of the retina is macula. It is responsible for our detailed vision. The centre of the macula is called the fovea which has anextensive role in detailed and coloured vision [Kishi, 2015].
- Optic nerve - The eye's blind spot is a result of the absence of photoreceptors in the area of the retina where the optic nerve leaves the eye. The cable network of optic nerve connect as a bridge subjoining eye to the brain and is composed of 1. 2 million nerve fibers. Optic nerve is also known as cranial nerve 2, which transmits the visual information from the retina to the brain [Reeves and Taylor, 2004; De Moraes 2013].
- Optic disc - The optic disk is the spot on the retina where the optic nerve leaves the eye. There are lack of sensory cells here, creating a blind spot [Quigley *et al.*, 1990].
- Vitreous humor - The Vitreous Humor is gel like substance which contributes to the shape of the eyeball and fills the space between lens and retina. It receives the nutrition from retinal vessels, ciliary body, aqueous and mainly supports to maintain the eye shape [Monteiro *et al.*, 2015].
- Aqueous Humor - The Aqueous Humor is the watery region in the eye ball. It is dissociated into two distinct regions, the anterior chamber forging into frontal region of the iris and the posterior chamber backing behind it. The canal of Schlemm drains water in to aqueous humor and is sometimes blocked off leading to the disease known as glaucoma [Goel *et al.*, 2010].
- Lens - The lens is a transparent layer behind the pupil made up of proteins and water. The lenspredominant purpose is to focus light by changing its shape. The ciliary body are muscles attached to the lens that help the lens change it shape to better focus light to the retina [Bassnett and Šikić, 2017; Miesfeld and Brown, 2019].

- Conjunctiva - The conjunctiva is a mucus layer that keeps the eye moist. Infections to this area are known as the popular “Pink Eye.” Lacrimal glands are found on the outer part of each eye and are producers of tears [Gipson, 2016; Miesfeld and Brown, 2019].

Conclusion

Eyes are a fundamental organ which detect and dredge the light and transform it into neurons as electro-chemical impulses. Nearly 90% of the genes in the human genome were expressed in eye’s tissue, hence eye is contemplated to have a crucial contribution in evolution. The phenotypic effects of minute genetic variations are apparent when many layers of amplification done in human visual system [Sheffield and Stone, 2011].

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